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New injectable melphalan formulations utilizing $(SBE)_{7m}$-β-CD or HP-β-CD

David Q. Ma [a,1], Roger A. Rajewski [b], Valentino J. Stella [a,b,*]

[a] *Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66047, USA*
[b] *Higuchi Biosciences Center for Drug Delivery Research, Lawrence, KS 66047, USA*

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Abstract

The objective of this work was to evaluate the potential of using $(SBE)_{7m}$-β-CD and HP-β-CD as enabling excipients to improve on the current melphalan injectable formulation. Melphalan is an anti-neoplastic agent formulated for parenteral use as a sterile, non-pyrogenic, freeze-dried powder. It is marketed by Glaxo-Wellcome as ALKERAN® for Injection (Alkeran). A major concern with melphalan therapy, other than its intrinsic cytotoxicity and biocompatibility, arises from its marginal aqueous solubility and chemical stability; thus, co-solvents are used in the current two-vial formulation. Because of the two-vial system, the product is also inconvenient to use. Two approaches to improve melphalan's formulation utilizing cyclodextrins, including the use of aqueous $(SBE)_{7m}$-β-CD or HP-β-CD solutions as the reconstitution diluents, and/or the use of $(SBE)_{7m}$-β-CD as a freeze-drying excipient in a melphalan formulation, are presented. Results showed that, when the cyclodextrins were used as diluents, the use of organic co-solvents can be eliminated and the shelf-life of the reconstituted melphalan greatly enhanced. When the freeze-dried melphalan/$(SBE)_{7m}$-β-CD formulation was prepared, the formulation was found to be stable; and a simplified one-vial delivery system was achieved. In conclusion, the parenterally safe β-cyclodextrins derivatives can provide promising alternatives and improved formulations for melphalan injectable and perhaps similar problematic drugs.

Keywords: Cyclodextrins; Sulfobutyl ether-β-cyclodextrins; 2-Hydroxypropyl-β-cyclodextrin; Melphalan; Co-solvents; Complexation.

1. Introduction

The objective of this study was to evaluate the potential of using aqueous $(SBE)_{7m}$-β-CD and HP-β-CD solutions as alternative delivery vehicles for melphalan injectable products. Melphalan is an antineoplastic compound effective in the treatment of various cancers (Anonymous, 1997). It is currently marketed as a freeze-dried product along with a second vial containing a co-solvent diluent. A major problem with melphalan therapy arises from its marginal aqueous solubility and instability on reconstitution and dilution. The

* Corresponding author. Tel.: +1-785-864-3755; fax: +1-785-864-5738.
E-mail address: stella@hbc.ukans.edu (V.J. Stella)
[1] Present address: 3M Pharmaceuticals, 3M Center, Building 260-4A-04, St. Paul, MN 55144, USA.

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two-vial system currently used is inconvenient and the use of the co-solvents potentially contributes to the side effects of the therapy (Medlicott et al., 1998).

Specifically, the current lyophilized formulation of melphalan is marketed as a sterile, non-pyrogenic, freeze-dried powder by Glaxo-Wellcome under the brand name ALKERAN® for Injection (Alkeran). Each single dose vial contains melphalan hydrochloride equivalent to 50 mg melphalan and 20 mg povidone. Alkeran is reconstituted using the sterile diluent provided, which contains 0.2 g sodium citrate, 6.0 ml propylene glycol, 0.52 ml ethanol (96%), and Water for Injection to a total of 10 ml. Alkeran is reconstituted by rapidly injecting 10 ml of this diluent directly into the vial of lyophilized powder, and then shaking immediately and vigorously until a clear solution is obtained. Rapid addition of the diluent followed by immediate vigorous shaking is important for proper reconstitution. This solution must then be diluted to the proper dose with saline (0.9% sodium chloride for injection; U.S.P.), to a concentration not greater than 0.45 mg/ml. Intravenous administration of the 110 ml of fully diluted Alkeran must be completed within 60 min of reconstitution due to the instability of the reconstituted and diluted solutions (Anonymous, 1997). Over periods as short as 30 min, a citrate derivative of melphalan produced by the reaction with melphalan has been detected in reconstituted material (Anonymous, 1997). Additionally, upon further dilution with saline, nearly 1% of the label strength of melphalan hydrolyzes every 10 min. Reconstituted Alkeran solutions cannot be refrigerated since a precipitate may form when the solutions are stored at 5°C.

The current melphalan formulation has several disadvantages. These can be summarized as follows.

1. *Reconstitution issues*. It is recommended that Alkeran should be vigorously shaken for 10 min to ensure complete dissolution of the lyophilized powder.
2. *Stability issues*. The active component of Alkeran, melphalan, is very unstable after reconstitution. The total use time allowed from the point of reconstitution is 60 min.
3. *Biocompatibility issues*. Exposure to the organic co-solvents and the drug itself can lead to irritation at the injection site. As with many other cytotoxic drugs (McEvoy, 1995), melphalan is administered by slow infusion. Tissue exposure to co-solvents and the drug at the injection site is inevitable, even though the blood flowing past this point will dilute the infusion fluid. The irritation effect of the fully diluted Alkeran co-solvents has been demonstrated in vitro by Medlicott et al. (1998), who showed that diluted Alkeran co-solvent caused significant cell disruption in a cell culture model.

Previous studies (Shiotani et al., 1994a,b; Rajewski et al., 1995) and reviews (Rajewski and Stella, 1996; Irie and Uekama, 1997; Thompson, 1997) show that two β-cyclodextrin derivatives, HP-β-CD (Encapsin™) and $(SBE)_{7m}$-β-CD (Captisol™), may be suitable for parenteral use due to their low systemic toxicity. Both $(SBE)_{7m}$-β-CD and HP-β-CD are excellent solubilizers and stabilizers for melphalan based on our previous studies (Ma et al., 1999a). As a result, the two cyclodextrin derivatives may be used to provide alternative and better formulations for the drug. This study examined the suitability of substituting the organic co-solvent diluents by cyclodextrin-based diluents and examined the possibility of formulating freeze-dried melphalan containing $(SBE)_{7m}$-β-CD as an enabling excipient as well as a bulking agent. While substituting the organic co-solvents in the current formulations by cyclodextrin diluents may minimize the side effects caused by the co-solvents, this approach may also provide greater stabilization for melphalan (Ma et al., 1999a).

2. Materials and methods

2.1. Materials

Alkeran was a generous gift from Glaxo-Wellcome. Melphalan was purchased from Sigma (St. Louis, MO). $(SBE)_{7m}$-β-CD (MW 2248.64, Lot TET 38-55) was provided by the Center for Drug Delivery Research (Lawrence, KS). HP-β-CD

(Encapsin™, MW 1338, Lot 92-3) was purchased from American Maize-Products Co. (Hammond, IN). Other chemicals used in this study were purchased from Fisher Co. (St. Louis, MO). All solvents were of high-performance liquid chromatography (HPLC) grade, and other reagents were of analytical grade.

2.2. HPLC assay

Quantitative determinations of melphalan were carried out by HPLC using a Beckman 110B pump, a Kratos spectroflow 757 absorbance detector operated at 260 nm, a Shimadzu CR601 integrator, a 20 μl injection loop, an ODS Hypersil column (5 μm, 150 × 4.6 mm, i.d.) and an ODS Hypersil guard column. The mobile phase consisted of a 50:50 methanol:acetate buffer (pH 4.7) with ionic strength adjusted to 0.1 M with NaCl. The retention time of the melphalan peak was 3.35 min at a flow rate of 1.8 ml/min.

2.3. Aqueous $(SBE)_{7m}$-β-CD or HP-β-CD used as the reconstitution diluents in a conventional delivery method

Solutions of 0.1 M $(SBE)_{7m}$-β-CD or HP-β-CD with 0.2 g/10 ml sodium citrate were prepared as diluents. Alkeran was reconstituted using the sterile co-solvent diluent provided with the Glaxo-Welcome formulation, the $(SBE)_{7m}$-β-CD diluent or the HP-β-CD diluent by rapidly injecting 10 ml of the diluents directly into the vial of lyophilized powder using a sterile needle and syringe. The vial was shaken immediately and vigorously until a clear solution was obtained. This 10 ml solution was diluted immediately by transferring it to 100 ml of 0.9% sodium chloride for injection, U.S.P. This reconstitution method is shown schematically in Fig. 1A. The solutions were kept in a 25°C water bath. Samples were removed at various time intervals, diluted to the proper concentration, and analyzed by HPLC for remaining melphalan. The pseudo-first-order rate constants, k_{obs}, for the degradation of melphalan were determined from the linear plots of the natural logarithm of the remaining melphalan concentration versus time.

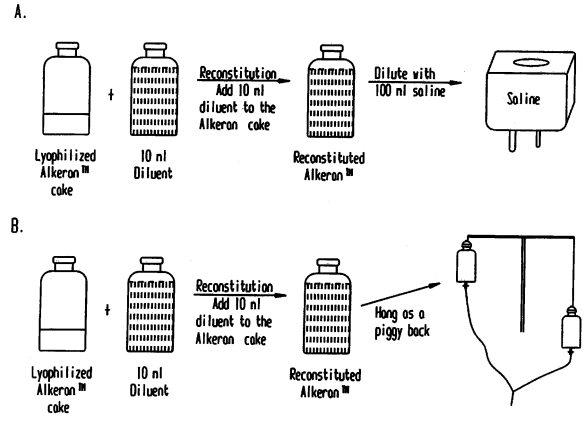


Fig. 1. The conventional (A) and piggy-back (B) reconstitution/administration methods.

The reconstitution time was estimated by recording the time from the moment that a diluent was injected into the vial of lyophilized powder until the point at which a clear solution was obtained after continuous vigorous manual-shaking of the vial. The reconstituted Alkeran was subsequently diluted by 100 ml of saline. The osmolarity of the fully diluted Alkeran solution was examined using an osmometer (μOsmeter™; Precision Systems Inc., Natick, MA).

2.4. Aqueous $(SBE)_{7m}$-β-CD or HP-β-CD used as the reconstitution diluents in a piggy-back delivery method

Alkeran was reconstituted in a manner similar to that already described, but not diluted with normal saline. The resultant solutions were kept at 25°C and samples were taken at various time intervals. The drug concentration remaining at the intervals was determined by HPLC. Values of k_{obs} were determined from the linear plots of the natural logarithm of the melphalan concentration remaining versus time. It was anticipated that such a vial could be piggybacked, as shown in Fig. 1B.

2.5. $(SBE)_{7m}$-β-CD used as the freeze-drying excipient in a melphalan formulation

A 100 ml solution of 0.1 M $(SBE)_{7m}$-β-CD containing 0.5 g melphalan was freshly prepared.

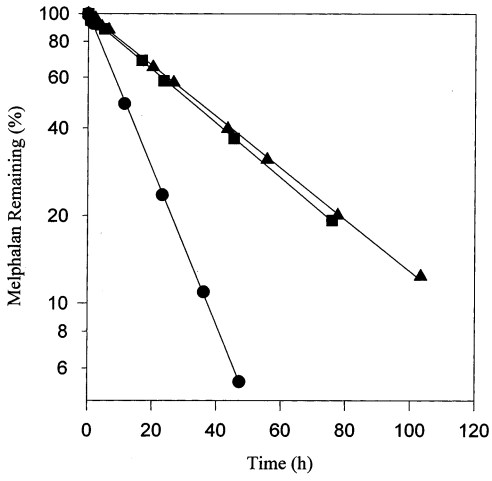


Fig. 2. A representative plot of melphalan degradation after it was reconstituted by various diluents (the organic co-solvents (●); the 0.1 M HP-β-CD diluent (■); and the 0.1 M $(SBE)_{7m}$-β-CD diluent (▲)) and further diluted by saline.

The final pH of the solution was 6.0. The solution was filtered through a 0.2 μm membrane, and 0.5 ml of the filtered solution was filled into 2 ml tubing glass vials. Freeze-drying was initiated by placing 100 vials directly on the freeze-dryer shelf. The shelf temperature was lowered to −50°C and kept at that temperature for 3 h to ensure complete solidification of the cakes. During the primary drying, the shelf temperature was first raised to −30°C (chamber pressure of 65 mTorr). The shelf temperature was then increased at increments of 2°C/h until −10°C was reached. The shelf temperature was then raised at increments of 5°C/4 h until 5°C. Finally, the shelf temperature was kept at 5 and 15°C for 4 h each. Secondary drying was carried out at 30°C for approximately 24 h at the same chamber vacuum. The melphalan concentration immediately before and after (on reconstitution) the freeze-drying cycle was complete was determined by the HPLC method. Residual moisture (freeze-dried solid) was measured by Karl–Fisher titration (KF Coulometer 684; Brinkmann).

Sealed melphalan/$(SBE)_{7m}$-β-CD freeze-dried products were stored at 5, 25, 37, and 50°C to examine the long-term stability of the products. At various intervals, samples were removed and diluted to the proper concentration with doubly distilled water at 5°C for analysis using the HPLC method. The time used to reconstitute the freeze-dried product was recorded. Corresponding moisture contents of the freeze-dried products at each sample interval were also measured by the Karl–Fisher method.

3. Results and discussion

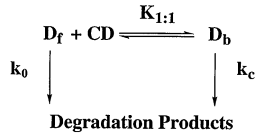
3.1. Aqueous $(SBE)_{7m}$-β-CD or HP-β-CD used as the reconstitution diluents in a conventional delivery method

Fig. 2 shows the normalized semi-log plot of percent melphalan remaining versus time after Alkeran was reconstituted by the co-solvents, $(SBE)_{7m}$-β-CD, and HP-β-CD diluents using the reconstitution method outlined in Fig. 1A, i.e. after the vials were reconstituted and the 10 ml vials diluted with 100 ml of normal saline. The time required for 10% Alkeran loss, $t_{90\%}$, from the kinetic studies is reported in Table 1. Both the

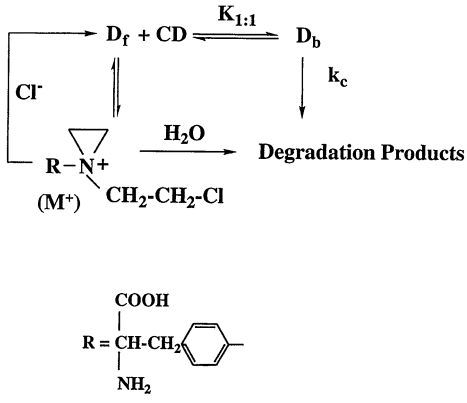
Table 1
Shelf-lives of the reconstituted Alkeren (melphalan injectable) product in the organic co-solvent supplied by the manufacture, or two cyclodextrin diluents ($n = 3$)

Delivery method	Diluent type $t_{90\%}$ (h)		
	Co-solvents	0.1 M $(SBE)_{7m}$-β-CD	0.1 M HP-β-CD
Conventional method[a]	1.69 ± 0.05	5.01 ± 0.10	5.01 ± 0.01
Piggy-back method[b]	7.48 ± 0.13	17.44 ± 0.43	8.48 ± 0.49

[a] The reconstitution and dilution following the instructions supplied by the manufacture.
[b] The melphalan product was reconstituted with 10 ml of the corresponding diluents and *not* further diluted.



Scheme 1. Melphalan degradation mechanism in the presence of cyclodextrins.



Scheme 2. Melphalan degradation mechanism in the presence of cyclodextrins and chloride ions.

$(SBE)_{7m}$-β-CD and HP-β-CD diluents improved the shelf-life of reconstituted melphalan threefold to over 5 h, compared with the co-solvent diluent (1.7 h).

Scheme 1 is commonly used to describe the degradation of drugs in the presence of cyclodextrins, where $K_{1:1}$ is the binding constant, D_f and D_b represent free and complexed drugs, and k_0 and k_c are the degradation rate constants of the drug in the free and complexed forms. The rate constant for the degradation of melphalan in the presence of the cyclodextrins, $k_{obs,calc}$, can be estimated by Eq. (1), using the known values of $[CD]_T$, $K_{1:1}$ and k_c determined previously (Ma et al., 1999a).

$$k_{obs,calc} = \frac{k_0 + k_c K_{1:1}[CD]_T}{1 + K_{1:1}[CD]_T} \quad (1)$$

The $K_{1:1}$ values for melphalan with $(SBE)_{7m}$-β-CD and HP-β-CD at 25°C and pH 6.0 were 360.0 and 381.6 M^{-1}, respectively, and the intrinsic degradation rate constants of the drug in its inclusion complex form (k_c) were 1.78×10^{-4} and 1.01×10^{-2} h^{-1}, respectively. $[CD]_T$ was 9.09×10^{-3} M after the melphalan formulation was diluted to a final volume of 110 ml. The predicted k_{obs} values of the diluted melphalan in the presence of $(SBE)_{7m}$-β-CD and HP-β-CD diluents were 2.2 and 2.0 h, respectively, which are less than half of the observed experimental values. This difference can be attributed to the presence of the chloride ions from the normal saline. Albert et al. (1979) have shown that the degradation of melphalan is inhibited by the addition of sodium chloride. To take the stabilizing effect of the chloride ion into consideration, an additional mechanism is proposed in Scheme 2 to describe Alkeran stability in the presence of both the cyclodextrins and chloride ions.

In Scheme 2, free melphalan is stabilized by chloride ions, which is contributing to the reverse reaction by competing with water molecules for attack on the ethyleneimmonium intermediate (M^+). Bound melphalan is stabilized by the cyclodextrin cavity and has an intrinsic reactivity of k_c. This model assumes that chloride ions, which are a small negatively charged species, are not present in the cyclodextrin cavities. Previous work (Hersey et al., 1986) has demonstrated that the binding constant of chloride ions with β-cyclodextrin cavities is extremely small ($K << 1$ M^{-1}). Thus, the degradation rate of the reconstituted melphalan, k'_{obs}, could be expressed by:

$$k'_{obs} = k_s f_{Df} + k_c f_{Db} \quad (2)$$

where k_s is the melphalan degradation rate constant in the same buffer with 0.9% NaCl at 25°C (determined experimentally), f_{Df} is the molar fraction of the free drug, and f_{Db} is the fraction of bound or complexed drug in the presence of the cyclodextrins. f_{Df} and f_{Db} can be calculated by knowing the $K_{1:1}$ values. The $K_{1:1}$ values of melphalan with the cyclodextrins were not affected by the change of ionic strength through the addition of sodium chloride under the experimental conditions (results not shown).

The predicted k'_{obs} values in the presence of the $(SBE)_{7m}$-β-CD and HP-β-CD diluents using Eq. (2) were 0.0251 and 0.0306 h^{-1}, which correspond to shelf-lives of 4.2 and 3.4 h, respectively. The predicted values are significantly closer to the experimental results than those predicted previ-

ously. The experimental $t_{90\%}$ value of the diluted Alkeran, when the organic co-solvent diluent was used, was longer than the use time recommended (60 min) by the manufacturer (Anonymous, 1997). This may be because it was assumed that the drug was 100% intact prior to reconstitution and did not take into account any drug degradation during storage of the product. Loss of the drug before reconstitution could result in a shorter $t_{90\%}$ than the experimental values from the kinetic results.

Table 2 shows the time to completely reconstitute the Alkeran freeze-dried cake by the diluents. Although the reconstitution time will vary with the vigour of shaking, the method used here was similar to that used in a practice setting. Every attempt was made to perform this task in a reproducible manner. The HP-β-CD diluent required a longer reconstitution time than the organic co-solvents or $(SBE)_{7m}$-β-CD. In the case of the organic co-solvent diluent, it was difficult to determine whether the lyophilized powder was completely dissolved, due to numerous air bubbles generated by the vigorous shaking. Transient sonication of the vials was used to clear the air bubble disturbance in order to determine whether the lyophilized cakes were completely dissolved. No transient sonication was necessary in the case of the cyclodextrin diluents and clear solutions were obtained immediately after the shaking stopped. Based on the presented information, the reconstitution time needed for the formulation was in the order of $(SBE)_{7m}$-β-CD ≤ co-solvent < HP-β-CD.

Table 2
Alkeran reconstitution time in the supplied co-solvent diluent, 0.1 M $(SBE)_{7m}$-β-CD or 0.1 M HP-β-CD, and the apparent tonicity values after dilution with normal saline

	Diluent type		
	Co-solvent	0.1 M $(SBE)_{7m}$-β-CD	0.1 M HP-β-CD
Alkeran reconstitution time (min)			
Trial 1	2	1.5	11
Trial 2	6	5	6
Trial 3	2	2	7
Average	3.3 ± 2.3	2.8 ± 1.9	8 ± 2.6
Osmometer readings[a]			
Average (n = 3)	1219 ± 1.0	347 ± 2.6	301 ± 2.0

[a] The readings were taken after Alkeran was reconstituted by the corresponding diluents (10 ml) and further diluted by 100 ml normal saline.

The osmolality of the diluted solutions was examined using a μOsmometer™. An isotonic saline solution was used for calibration. The instrument gave an isotonic reading of 299. In Table 2, reconstituted Alkeran was isotonic with the HP-β-CD diluent and slightly hypertonic with the $(SBE)_{7m}$-β-CD diluent at the chosen cyclodextrin diluent concentration. Since human serum has a toxicity range from 220 to 480, the solution toxicities of the diluted Alkeran/cyclodextrin solutions were in the acceptable range. This was consistent with the results from the study by Medlicott et al. (1998), which showed that the cell culture was not disturbed at the same cyclodextrin concentrations. The osmometer reading of 1219 from the diluted solution with the co-solvents was not comparable with other readings. This is because the presence of the organic co-solvents in the solution disturbed the freezing point of the aqueous solution. However, the study by Medlicott et al. (1998) showed that the use of the diluted organic co-solvents did cause cell disturbance.

No precipitation of the reconstituted Alkeran upon refrigeration was observed when using the $(SBE)_{7m}$-β-CD or HP-β-CD as the diluents. Binding constants between a drug and the cyclodextrins normally increase with decreasing temperature. Even though the solubility of the free drug might decrease at a lower temperature, precipitation was avoided due to increased binding constants, which would result in an increase of the fraction of the drug in the complexed form.

3.2. Aqueous $(SBE)_{7m}$-β-CD or HP-β-CD used as the reconstitution diluents in a piggy-back delivery method

The idea of this piggy-back method was to keep the reconstituted Alkeran (melphalan) from being further diluted by the 100 ml saline. Upon deliv-

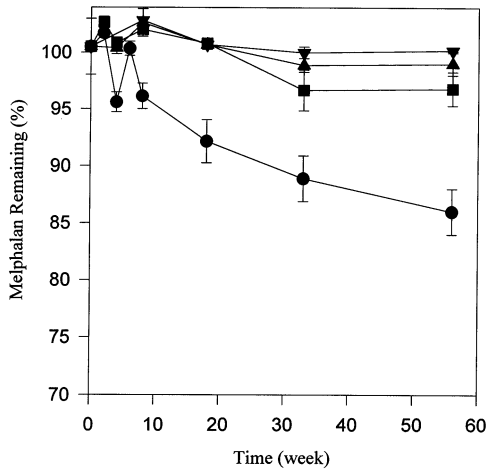


Fig. 3. Remaining melphalan in the long-term stability study of the lyophilized melphalan/$(SBE)_{7m}$-β-CD product at 50°C (●), 37°C (■), 25°C (▲), and 5°C (▼).

ery, this reconstituted melphalan could function as a piggy-back and be co-infused through a common line with the 100 ml saline (Fig. 1B). It is well known that the stabilization potential of cyclodextrins for drugs depends greatly on the cyclodextrin concentration (Loftsson and Brewster, 1996), with greater cyclodextrin concentration leading to a longer drug shelf-life. This method keeps the drug in a more concentrated cyclodextrin environment. The drug would be diluted only immediately before infusion into patients. Thus, the reconstituted product was expected to have a longer shelf-life than in a diluted cyclodextrin solution, as in the conventional delivery method.

As shown in Table 1, this method indeed greatly improved the shelf-life of reconstituted Alkeran with all diluents. The longest shelf-life was observed with the $(SBE)_{7m}$-β-CD diluent, which gave a $t_{90\%}$ of 17.4 h. The shelf-life of the reconstituted melphalan in its organic co-solvents was enhanced, and is consistent with our previous observation (Ma et al., 1999a) that melphalan is more stable in a less polar solvent.

Since chloride ions were not present in the reconstituted Alkeran vial, Eq. (1) predicted the shelf-lives of reconstituted Alkeran in the $(SBE)_{7m}$-β-CD and HP-β-CD diluents to be 14.6 and 7.0 h, respectively. The predicted values agree reasonably well with the experimental results. The greater stability of Alkeran in the presence of the $(SBE)_{7m}$-β-CD diluent than in that of HP-β-CD was due to lower melphalan intrinsic reactivity in $(SBE)_{7m}$-β-CD (Ma et al., 1999a).

3.3. $(SBE)_{7m}$-β-CD used as the freeze-drying excipient in a melphalan formulation

The freeze-drying cycle used in this study is effective and efficient. The freeze-dried cakes retained their elegance and no collapse was observed. It was found that the melphalan remained amorphous in the frozen melphalan/$(SBE)_{7m}$-β-CD solution. The maximally freeze-concentrated glass transition temperature, T'_g (-28.5°C), of the frozen $(SBE)_{7m}$-β-CD solution did not change with the addition of melphalan. This lack of effect on the T'_g of $(SBE)_{7m}$-β-CD is probably due to an excess amount of the cyclodextrin material in the formulation. The lack of change in the remaining melphalan concentration immediately before and after lyophilization indicated that no observable drug loss occurred during freeze-drying.

The moisture content of the freeze-dried products remained at $4.5 \pm 0.5\%$ throughout the long-term stability study period. Fig. 3 shows the long-term stability of the freeze-dried products. Degradation was not significant over approximately one year at 5, 25, and 37°C, but some loss was observed in the batch at maintained 50°C. This implies that the moisture in the melphalan/$(SBE)_{7m}$-β-CD-lyophilized cakes at 4.5% had a minimal effect on melphalan degradation. The results also suggest that a lyophilized stable formulation of melphalan/$(SBE)_{7m}$-β-CD is possible. This approach not only eliminates the use of organic co-solvents, but also simplifies a two-vial delivery system to a one-vial system. The reconstitution time of the freeze-dried products using normal saline was found to be less than 10 s. This may be attributed to the porous amorphous cake structures formed during the freeze-drying cycle (Ma et al., 1999b).

4. Conclusions

The employment of the cyclodextrin derivatives $(SBE)_{7m}$-β-CD and HP-β-CD as the alternative delivery vehicles for an injectable melphalan formulation was shown to be quite promising. When cyclodextrin solutions were used as initial diluents, the use of organic co-solvents was avoided, which should result in more biocompatible dosage forms. These formulations also greatly enhanced the shelf-life of the drug after reconstitution. After reconstitution and dilution with normal saline, shelf-lives of about 5 h were observed when using the $(SBE)_{7m}$-β-CD and HP-β-CD, versus 1.7 h with the co-solvent diluent. In the so-called piggyback delivery method, the shelf-lives were extended to 17.4, 8.5 and 7.5 h with the $(SBE)_{7m}$-β-CD, HP-β-CD, and co-solvent diluents, respectively. The predicted shelf-lives in the presence of the cyclodextrin diluents using known $K_{1:1}$ and k_c values from an earlier study agreed reasonably well with the experimental results. This implies that knowing $K_{1:1}$ and k_c could assist in the rational design of formulations containing the cyclodextrins. A freeze-dried melphalan/$(SBE)_{7m}$-β-CD formulation was successfully prepared and was found to be very stable over a period of 1 year. This formulation approach simplifies the two-vial delivery system to a one-vial system as well as having the other advantages noted in this work.

Acknowledgements

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