Cephalothin Stability in Normal Saline, Five Percent Dextrose in Water, and Dianeal® Solutions

Scott E. Walker, Thomas W. Paton and Dimiteros G. Oreopoulos

ABSTRACT

The stability of cephalothin sodium (1 g) in polyvinyl chloride minibags containing 50 mL of 0.9% normal saline, 5% dextrose in water (D5W) or 500 mg in 2L of Dianeal® (containing 1.5% or 4.5% dextrose) was evaluated using a validated, stability-indicating, liquid chromatographic method during 30 days storage at 4°C and room temperature (23°C). Physical inspections and pH determinations also completed on each of the 11 study days during the 30 day storage period.

During the 30-day study period all solutions lost more than 10% of the initial cephalothin concentration. In D5W and saline solutions stored at 4°C, 10% of the initial concentration was lost within 17 days, and at room temperature 10% was lost within one day. In Dianeal®, regardless of the dextrose concentration, solutions stored at 4°C, lost 10% of the initial concentration within eight days, and at room temperature 10% was lost within one day. During the 30-day study period the pH decreased in every solution. However, the decrease was less than 1.0 of a pH unit. The colour of solutions stored at room temperature gradually changed during the study period, becoming a deeper yellow. Solutions stored at °C remained clear and colourless throughout the study period.

We conclude that cephalothin solutions (1 g/50 mL of D5W or normal saline) stored at 4°C for seven days followed by 12 hours storage at room temperature will retain 90% of the initial cephalothin concentration, whereas, cephalothin concentrations stored in Dianeal® (500 mg/2L) at 4°C will lose approximately 8% of the initial concentration after only three days storage at 4°C followed by an additional 12 hours storage at room temperature.

Key Words: *cephalothin, stability*

Can J Hosp Pharm 1992;45:237-42

RÉSUMÉ

On a déterminé la stabilité de la céfalotine sodique (1 g) dans des mini-sacs de chlorure de polyvinyle renfermant 50 mL de chlorure de sodium à 0,9%, 5% de dextrose dans de l'eau (D5E) ou 500 mg dans 2 L de Dianeal® (1,5 ou 4,5% de dextrose). La stabilité du produit a été établie par une technique de chromatographie en phase liquide éprouvée, après 30 jours d'entreposage à 4°C et à température ambiante (23°C). On a procédé à un examen physique du produit, dont on a également mesuré le pH chacun des 11 jours d'essai, durant la période d'entreposage de 30 jours.

Pendant l'entreposage, la concentration originale de céfalotine de chaque solution a diminué de plus de 10%. La perte s'est manifestée au bout de 17 jours dans les solutions de D5E et NaCl 0,9%, à 4°C, alors qu'on l'a enregistrée au bout d'une journée, à température ambiante. Dans le Dianeal', peu importe la concentration de dextrose, la concentration initiale de la solution diminue de 10% au bout de huit jours, à 4°C, et au bout d'une journée, à température ambiante. Le pH de chaque solution a baissé durant la période d'entreposage de 30 jours. Toutefois, la baisse était inférieure à 1,0 unité. Les solutions gardées à température ambiante ont changé peu à peu de couleur durant l'étude et pris une teinte jaune plus prononcée. Les solutions conservées à 4°C sont restées translucides et incolores durant toute la période d'étude.

On en conclut que les solutions de céfalotine (1 g/50 mL de D5E ou de NaC1 0,9% gardées sept jours à 4°C puis 12 heures à température ambiante maintiennent 90% de leur concentration initiale de céfalotine, tandis que les solutions de Dianeal® (500 mg/2L) perdent approximativement 8% de leur concentration initiale après trois jours seulement d'entreposage à 4°C, puis 12 heures à température ambiante.

Mots clés: *céfalotine, stabilité*

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Acknowledgements: This study was funded by the Department of Pharmacy, Sunnybrook Medical Centre. The authors would like to acknowledge the technical assistance of Bettina Fong, a Pharmaceutical Technology Student from Seneca College.

INTRODUCTION

Cephalothin is a beta-lactamase-resistant broad spectrum cephalosporin which has been available for many years. Even though a number of studies describing the stability of cephalothin have been published[1-5] very few describe cephalothin stability under conditions of use normally encountered by those preparing the medication for patients and no study has evaluated cephalothin stability in Dianeal® solutions. In Canada, the manufacturer (Eli Lilly Canada Inc.) recommends that freshly reconstituted vials stored under refrigeration be used within 72 hours

and that solutions prepared for IV infusion be used within 24 hours[6]. In the United States, the manufacturer (Eli Lilly & Co.) recommends that reconstituted solutions stored at 4°C be used within 96 hours or within 24 hours if stored at room temperature[7]. Therefore, while not specifically reported in any study, it might reasonably expect that dilute solutions of cephalothin would be sufficiently stable under refrigeration to permit storage for up to 72 to 96 hours. However, in our continuous ambulatory peritoneal dialysis program, patients receiving 2L Dianeal® complained of irritation during dwells when the instilled solutions contained 500 mg of cephalothin and had been stored for only 24 hours at room temperature.

Therefore, the purpose of this investigation was to evaluate the chemical stability of cephalothin in Dianeal® solutions, normal saline and five percent dextrose in water (D5W) and to determine reasonable expiry dates for cephalothin in these solutions.

METHODS

Assay Validation

Following the development of the chromatographic system for cephalothin, the suitability of this system for use as a stability-indicating assay method was tested by accelerating the degradation of cephalothin. Cephalothin sodium, 500 mg (Keflin®: Eli Lilly Canada; lot #35228) was dissolved in 25 mL of water, placed in a 30 mL multidose vial (Bencard; Division of Beecham Laboratories) and incubated in a water bath at 61°C protected from light for 390 minutes. Samples were drawn just prior to incubation and at 15, 30, 45, 60, 75, 130, 169, 195, 227, 257, 290, 335, and 390 minutes. This study was terminated at 390 minutes because at this point less than 5% of the initial cephalothin concentration remained. Chromatograms were inspected for the appearance of additional peaks and the cephalothin peak was compared between samples for changes in concentration, retention time and peak shape.

Following this first phase of evaluation and validation, the accuracy and reproducibility of standard curves was tested over five days and system suitability criteria (theoretical plates, tailing and retention time) were developed to ensure consistent chromatographic performance. On each day, 30 mg of cephalothin sodium powder (Keflin®: Eli Lilly Canada — lot #35228) was dissolved in 100 mL of water resulting in a final concentration of 300 mg/L. Samples of this stock solution were diluted with water to obtain standards with final concentrations of 50, 100, 150, and 200 mg/L. These standards served to construct a standard curve. Each sample was chromatographed in duplicate. As well, a 250 mg/L sample of cephalothin hydrochloride was prepared on each day, chromatographed and its concentration determined and compared to its known concentration. Intra-day error was assessed by the coefficient of variation of the peak area for six replicates each chromatographed in duplicate.

Stability Study

On study day zero, 1 g vials of cephalothin sodium (Keflin®; Eli Lilly Canada — lot #35228) were each reconstituted with 10 mL of sterile water for injection. The contents of each vial (1 g) was added to each of 17 - 50 mL polyvinyl chloride (PVC) bags of normal saline (Baxter Canada) and 17 - 50 mL PVC bags of D5W (Baxter Canada). Four normal saline bags and four D5W bags were stored at room temperature (23°C) and all others were stored at 4°C. An additional 500 mg of cephalothin was added to each of four 2L PVC bags of Dianeal® (1.5% dextrose; 4.5% dextrose : Baxter Canada). One 2L bag of each strength of Dianeal® (1.5% and 4.5% dextrose) was stored at room temperature (23°C) and the remaining two bags were stored at 4°C.

Cephalothin Analysis

Solutions containing 1 g of cephalothin sodium were stored at 4°C or 23°C, and samples were drawn on each of nine study days (0, 1, 3, 8, 10, 14, 18, 24, and 30). Dianeal® solutions containing 500 mg of cephalothin stored at 4°C or 23°C were sampled on each of 11 study days (0, 1, 2, 3, 4, 8, 10, 14, 18, 24, and 30). Fifteen microlitres of each sample were directly chromatographed in duplicate.

Due to the 80-fold difference in nominal concentration between Dianeal® solutions and normal saline and D5W solutions, two independently prepared standard curves were constructed on each study. The standard curve constructed for the D5W and normal saline samples was prepared by dissolving 10 mg, 20 mg and 30 mg of cephalothin sodium powder (Keflin®: Eli Lilly Canada — lot #35228) in 1 mL of distilled water. Two additional accurate weights of 15 mg and 25 mg of cephalothin were also were dissolved in 1 mL of water and used to calculate accuracy. Three microlitres of each of these standards and a blank were directly chromatographed in duplicate.

The standard curve constructed for Dianeal® samples was prepared by dissolving 30 mg of cephalothin sodium powder (Keflin; Eli Lilly Canada — lot #35228) in 100 mL of distilled water. Samples of this stock solution were then further diluted with water to obtain standards with final concentrations of 50, 100, 150, and 200 mg/L. An additional standard of 250 mg/L was also prepared and chromatographed on each day and was used to calculate accuracy. Fifteen microlitres of each of these standards and a blank were directly chromatographed in duplicate.

The liquid chromatographic (LC) system consisted of an isocratic solvent delivery pump (Spec-

tra Physics: Model 4200) which pumped a mixture of acetonitrile (Fisher: cat. #A998), and 0.05 molar potassium phosphate monobasic (Fisher: cat. #P286) through a 25 cm x 4.2 mm reversed-phase C-18, 5 μm column (Ultrasphere ODS, #235329; Beckman) at 2.0 mL/min. The ratio of acetonitrile to phosphate buffer was 20:80 and was held constant during a chromatographic run. On each day the strength of the mobile phase was prepared to achieve a retention time for cephalothin between 270 and 360 seconds. Samples were introduced into the LC system using an autoinjector (WISP 715; Waters).

The column effluent was monitored with a variable wavelength ultra-violet detector (Applied Biosystems; Model 759A) at 254 nm. A signal from the detector was integrated and recorded with a chromatographic integrator (Spectra Physics: Model 4240). The area under the cephalothin peak at 254 nm was reported and used to calculate the cephalothin concentration.

Standard curves were prepared daily, as previously described. The peak area of the cephalothin peak was subjected to least squares regression analysis and the actual cephalothin concentration, from each solution, was interpolated from these curves and recorded to the nearest 0.01 mg/mL.

Physical Evaluation

On each of the study days, the pH of one of each of the solutions stored at each temperature was measured and recorded to the nearest 0.001 of a pH unit. The pH meter (Fisher: Accumet-model 925) was equipped with a microprobe glass body electrode (Fisher: cat #13-639-280) and was standardized each day with two commercially available buffer solutions.

On each of the study days, each container was also inspected visually for colour and clarity. Visual particulate matter inspection was performed against a black and white background.

Data Reduction and Statistical Analysis

Means were calculated for analyses completed in duplicate or triplicate. Reproducibility was assessed by the coefficient of variation (CV: standard deviation divided by the mean). Mean results from different days of an identical test were compared statistically by least squares linear regression to determine if an association existed between the observed result and time. Log-linear and linear-linear fits for the data from the accelerated degradation study (61°C) were compared for goodness of fit by the Maximum Likelihood Method of Box and Cox[8,9]. Analysis of variance and the least significant difference multiple range test was used to compare differences between temperature, and/or solutions for similar analytical tests. The five percent level was used as the apriori cut-off for significance and all reference to significance refers to this level.

Cephalothin concentrations were considered "acceptable" or "within acceptable limits" if the concentration on any day of analysis was not less than 90% of the initial (day-zero) concentration.

RESULTS

Accelerated Degradation and Assay Validation

When dissolved in water and heated at 61°C, cephalothin degraded in an apparent first order fashion with a half-life of 88 minutes (Figure 1). After 390 minutes, less than 5% of the initial cephalothin concentration remained and several degradation products could be observed (Figure 1). The results of

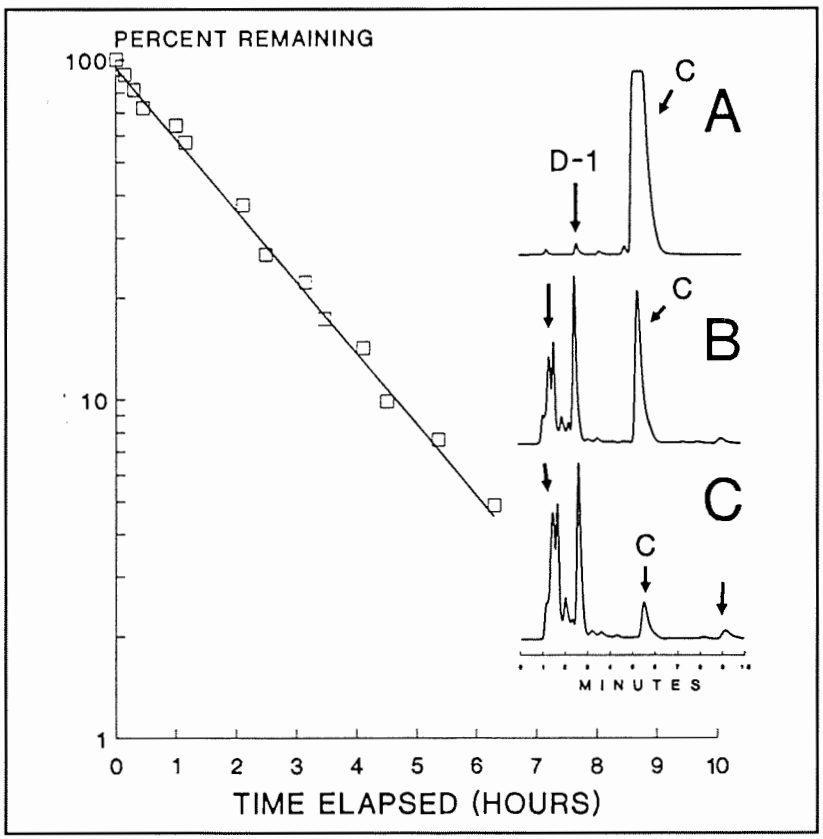

Figure 1: Rate of loss of cephalothin during the accelerated study at 61°C. Log-linear coefficient of determination: $r^2 = 0.995$ versus linear-linear: $r^2 = 0.8897$. As the cephalothin concentration declined, several degradation products, including the major degradation product marked "D-1", could be observed in chromatograms. Inset panels A, B and C represent chromatograms of samples drawn at time zero, 195 minutes and 390 minutes, respectively. The peak marked "C" represents cephalothin; D-1 represents the major degradation product and arrows indicate other degradation products.

this investigation, that is, predictable degradation and chromatographic separation of degradation products and cephalothin, indicated that this analytical method was stability-indicating[10-12].

Analysis of accuracy and reproducibility evaluations indicated that the cephalothin concentration was measured accurately. Recovery, based on the mean of duplicate determinations was within 95-103% of the theoretical concentrations of 15 mg/mL, 25 mg/mL and 250 mg/L. Analytical reproducibility, within a day (as measured by CV determined on six replicates) averaged 1%, regardless of concentration. Inter-day reproducibility, assessed by analysis of the concentration determined on five consecutive days following storage at 4°C can be confounded by degradation. However, at 250 mg/L the CV averages 2%, and at 15 mg/mL and 25 mg/mL the CV averaged 2.3%. This indicates that differences of 7% or more can be confidently detected with acceptable error rates[13]. System suitability criteria were developed based on daily calculations of theoretical plates, tailing, retention time and accuracy observed during the validation period and were used to ensure continued chromatographic performance during the study period. Quality control samples (250 mg/L; 15 mg/mL and 25 mg/mL) run during the study to determine accuracy averaged 97.46 +/- 1.10%.

Cephalothin Stability Study

Over the 30 day study period there was a significant trend for cephalothin concentrations to decrease in normal saline and D5W solutions (Table I) at 4°C and 23°C. More than 10% of the initial cephalothin concentration was lost after one day of storage at room temperature and 18 days storage at 4°C. After 30 days of storage, normal saline and D5W solutions stored at 4°C retained approximately 84% of the initial cephalothin concentration whereas solutions stored at room temperature retained less than 5% (Tables I and III). Dianeal® solutions also degraded quickly at room temperature such that less than 10% of the initial concentration remained after 30 days compared to about 66% in solutions stored at 4°C (Tables II and III).

The relative size of additional peaks observed during the accelerated degradation studies, increased during the 30-day study period. Although the apparent concentration of these degradation products increased during the study as cephalothin concentrations declined, the concentration of these products was not quantified. No significant difference in degradation rate was apparent between normal saline or D5W solutions stored at the same temperature (Tables I and III). Similarly, both Dianeal® solutions, regardless of the dextrose concentration, demonstrated similar degrees of degradation at the same storage temperature. However, temperature (4°C vs 23°C) did have a significant effect on degradation rate for all solutions, such that a greater rate of loss in cephalothin concentration was observed at room temperature (Table III).

During the 30-day study period, pH decreased in all solutions stored at 4°C and 23°C. However, all changes were less than one pH unit and there was no apparent depen-

Table I: Mean[a] Cephalothin Concentrations (1 g/50 mL) of Normal Saline (NS) or 5% Dextrose in Water

Study Day	1 g/50 mL D5W @ 4°C	1 g/50 mL D5W @ RT	1 g/50 mL NS @ 4°C	1 g/50 mL NS at RT
0[c]	16.90 ± 0.32	15.82 ± 0.27	15.81 ± 0.13	15.81 ± 0.23
1	16.76 ± 0.70	15.12 ± 0.41	16.32 ± 0.36	14.28 ± 0.38
3	16.92 ± 0.51	11.25 ± 0.31	16.19 ± 0.23	12.10 ± 0.31
8	15.80 ± 0.90	6.92 ± 0.12	16.76 ± 0.33	7.26 ± 0.30
10	UC[b]	UC[b]	16.49 ± 0.25	3.71 ± 0.09
14	16.09 ± 0.81	3.65 ± 0.19	16.68 ± 0.53	3.71 ± 0.09
18	15.86 ± 1.25	1.97 ± 0.15	17.09 ± 0.56	2.12 ± 0.11
24	14.14 ± 0.47	0.69 ± 0.04	14.07 ± 0.32	0.76 ± 0.07
30	14.16 ± 0.40	0.48 ± 0.05	13.29 ± 0.65	0.54 ± 0.06
% remaining (Day 30/Day 0)*100	83.79	3.03	84.06	3.42
First Order Rate Constant (day^{-1})	0.00612	0.12218	0.00553	0.11743
T_{90} (days)[d]	17.17	0.86	18.99	0.89

a Concentrations are mean of three PVC minibags, each determined in duplicate on each study day. Concentrations are reported (mg/mL) ± standard deviation.
b UC indicates unacceptable chromatography.
c Concentrations reported on day zero appear to be less than theoretical (20 mg/mL) due to the addition of water which occurs when adding reconstituted drug and due to manufacture's overfill of the minibag.
d T_{90} indicates the time to reach 90% of the initial concentration.

Table II: Mean[a] Cephalothin Concentrations (mg/L) in Dianeal® [1.5% amd 4.5% Dextrose]

Study Day	1.5% Dextrose at RT	1.5% Dextrose at 4°C	4.5% Dextrose at RT	4.5% Dextrose at 4°C
0[b]	284.92	281.42	281.00	276.60
1	257.65	266.53	254.82	264.30
2	241.77	265.79	238.60	263.76
3	223.40	269.72	225.40	267.53
4	203.50	257.43	201.46	254.51
8	155.70	248.05	153.25	245.74
10	129.69	233.49	128.56	232.33
14	100.35	231.31	98.94	213.21
18	71.55	218.90	70.99	215.71
24	42.54	199.07	40.69	196.57
30	27.13	185.91	24.96	185.81
% remaining (Day 30/Day 0)*100	9.52	66.06	8.89	67.18
First Order Rate Constant (day^{-1})	0.07784	0.01315	0.07978	0.01326
T_{90} (days)[c]	1.35	7.98	1.32	7.92

a Concentrations (mg/L) are the average of two duplicates chromatographed on each study day.
b Concentrations reported on day zero appear to be less than theoretical (20 mg/mL) due to the addition of water which occurs when adding reconstituted drug and due to manufacture's overfill of the Dianeal® bag.
c T_{90} indicates the time to reach 90% of the initial concentration.

Table III: Mean Cephalothin Degradation Rate Constants Based on Solution Type and Temperature

	First Order Rate Constant [day^{-1}]	T_{90} [days][a]	% remaining after		
			12 hours	24 hours	7 days
Normal Saline and D5W Solutions					
at 4°C	0.00583	18.01	99.71	99.42	96.00
at 23°C	0.11981	0.87	94.19	88.71	43.23
Dianeal® Solutions					
at 4°C	0.01321	7.95	99.34	98.69	91.17
at 23°C	0.07881	1.33	96.14	92.40	57.60
37°C [estimated]	0.19255	0.55	90.82	82.49	25.98

a T_{90} indicates the time at which 90% of the initial concentration is reached.

dency on solution type or storage temperature. The colour gradually changed during the study period to yellow and all solutions had a sulphurous odour. Solutions stored at room temperature changed colour faster and more rapidly developed the sulphurous odour than those stored at 4°C.

DISCUSSION

Statistical analysis of the cephalothin concentration time data in this study was limited to least squares log-linear regression because demonstration of a trend for the concentration to decrease was considered more important than demonstrating a statistical difference in concentration between any two days. In fact, the random fluctuations in concentration around a line of 'best fit' are not of practical importance and should be considered 'noise' or experimental error. Least squares log-linear regression indicated that a 10% loss in the initial cephalothin concentration was observed after less than one day of storage at room temperature in normal saline or D5W; and at 4°C, 10% of the initial concentration would be lost in these solutions within 18 days (Tables I and III). However, a recommended expiry date must consider that a prepared product will be stored for a period of time at both 4°C and room temperature. Using the first order degradation rates observed in this study (Table III), it is estimated that greater than 90% of the initial cephalothin concentration would remain after seven days of storage at 4°C followed by an additional 12 hours storage at room temperature. Similarly, 10% of the initial cephalothin concentration was lost after one day of storage at room temperature in Dianeal® solutions and at 4°C, 10% of the initial concentration would be lost within eight days (Table II and III). However, after consideration of the fact

that the product will be brought to room temperature or even 37°C before being instilled into a patient, using the degradation rates observed in this study (Table III) it is estimated that greater than 90% of the initial cephalothin concentration would remain after four days storage at 4°C followed by an additional 12 hours storage at room temperature. Since the degradation rate changes by a factor of roughly 2.4 for every 10°C shift in temperature (Table III), calculations predict that bags of Dianeal® containing cephalothin, would lose more than 10% of their initial concentration if they spent more than 12 hours at 37°C, or were stored at room temperature for 24 hours and then spent more than two hours at 37°C before being instilled into the patient. Therefore, if patients are to receive a 24 hour supply of Dianeal® bags containing cephalothin, it is recommended that these bags be stored in the refrigerator and then be warmed to 37°C immediately prior to being instilled. While we have no specific information regarding the degradation products of cephalothin and their tendency to irritate peritoneal tissues, we suspect that the irritation reported by patients after using bags containing cephalothin that had been prepared only 24 hours before is related to the presence of degradation compounds within the bag.

We conclude that freshly reconstituted cephalothin powder used to prepare solutions of 1g/50 mL minibag of normal saline or D5W should not be stored for more than 12 hours at room temperature (23°C), but can be stored for up to 18 days at 4°C. However, after consideration of the fact that a minibag will be held at room temperature for some period of time prior to use, we recommend that an expiry date not exceeding seven days storage at 4°C be established. With such an expiry date, (which allows for up to an additional 12 hours of storage at room temperature) it is estimated that more than 90% of the initial cephalothin concentration will remain. We also conclude that solutions of 500 mg/2L of Dianeal® will retain more than 90% of the initial cephalothin content when stored for up to 24 hours at 23°C or for less than eight days when stored at 4°C. However, given that a Dianeal® bag is generally warmed to 37°C prior to instillation, we recommend that an expiry date not exceeding three days storage at 4°C be established. This expiry date allows for up to an additional six hours of storage at 37°C or 12 hours storage at room temperature. It is estimated that more than 92% of the initial cephalothin concentration will remain at the end of the expiry date.

These expiry dates must only be used after due consideration of sterility and the contamination rate of IV admixtures prepared in an IV additive program.

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