Concentration and Solution Dependent Stability of Cloxacilllin Intravenous Solutions

Scott E. Walker, Annie Dufour and John Iazzetta

ABSTRACT

The stability of cloxacillin at 5, 10, 20, 40, and 50 mg/mL in 5% dextrose in water (D5W) and normal saline (NS) solutions, stored at 4°C and 23°C was tested over an 18-day period. In addition to visual inspection and pH, the concentration of cloxacillin was determined by a stability-indicating liquid chromatographic method. Within and between days analytical error averaged less than 3%.

The osmolarity of cloxacillin concentrations ranging from 0 mg/mL to 50 mg/mL in 3 different diluents, NS, D5W, and sterile water (SW), was also tested. The osmolarity of solutions in SW was low (204 mOsm for the 50 mg/mL) while the osmolarity of NS and D5W solutions ranged from 285 to 432 mOsm for all concentrations between 5 and 50 mg/mL. Therefore, stability studies were conducted only on NS and D5W solutions.

At room temperature, all NS solutions retained more than 90% of their initial concentration for only 24 hours, whereas all D5W solutions, other than the 50 mg/mL solution, were more stable retaining 90% of the initial concentration for 4 days at room temperature. The 50 mg/mL solution developed a precipitate on the fourth day. After 18 days at room temperature, D5W solutions had between 20.27% remaining (50 mg/mL solution) and 47.9% (5 mg/mL solution, whereas the cloxacillin concentrations of NS solutions ranged from 21.5% (50 mg/mL) to 38.5% (5 mg/mL) remaining. Under refrigeration, D5W and NS solutions both retained more than 90% of the initial concentration for 18 days.

In conclusion, we recommend that cloxacillin solutions be stored for 7 days at 4°C and then allowed to stand for no more than 24 hours at room temperature. Based on the faster rate of degradation observed at room temperature for NS solutions, D5W is the preferred diluent.

Key Words: Cloxacillin, Concentration Dependent, Stability

RÉSUMÉ

La stabilité de la cloxacilline à 5, 10, 20, 40 et 50 mg/mL dans des solutions de dextrose à 5 % dans l'eau (D5W) et de soluté physiologique normal (NS), entreposées à 4 °C et à 23 °C, a été testée sur une période de 18 jours. Outre les inspections visuelles et à la vérification du pH, on a déterminé la concentration des solutions en cloxacilline, au moyen d'une épreuve de stabilité par chromatographie liquide. La marge d'erreur analytique pour une même journée ou entre deux journées, déterminée par une analyse d'échantillon répété, était inférieure à 3 % en moyenne.

L'osmolarité des concentrations de cloxacilline qui variaient entre 0 mg/mL et 50 mg/mL dans trois diluants différents, soit le NS, le D5W et l'eau stérile (SW), a aussi été mesurée. L'osmolarité des solutions de SW était faible (104 mOsm pour les concentrations de 50 mg/mL), comparativement à celle des solutions de NS et de D5W qui variait de 285 à 432 mOsm pour toutes les concentrations entre 5 et 50 mg/mL. Par conséquent, les études de stabilité n'ont été menées que pour les solutions de NS et de D5W.

À la température ambiante, toutes les solutions de NS ont conservé plus de 90 % de leur concentration initiale de cloxacilline pendant seulement 24 heures, contrairement aux solutions de D5W, sauf les solutions à 50 mg/mL, qui étaient plus stables et qui ont conservé 90 % de leur concentration initiale en cloxacilline durant 4 jours, à la température ambiante. Un précipité s'est formé dans la solution à 50 mg/mL, au quatrième jour. Après avoir été entreposées pendant 18 jours à la température ambiante, les solutions de D5W avaient conservé 20,27 % (solution de 50 mg/mL) et 47,9 % (solution de 5 mg/mL) respectivement de leurs concentrations en cloxacilline, alors que les solutions de NS n'en avaient conservé qu'entre 21,5 % (50 mg/mL) et 38,5 % (5 mg/mL), respectivement. Réfrigérées, les solutions de D5W et de NS ont toutes deux conservé plus de 90 % de leurs concentrations initiales en cloxacilline pendant 18 jours.

En conclusion, nous recommandons d'entreposer les solutions de cloxacilline pendant 7 jours, à une température de 4 °C et de ne pas les laisser à la température ambiante pendant plus de 24 heures. Étant donné la vitesse de dégradation plus grande des solutions de NS à température

Scott E. Walker, MScPhm, is Coordinator, Research and Quality Control, Department of Pharmacy, and Division of Clinical Pharmacology, Sunnybrook Health Science Centre and Associate Professor, Faculty of Pharmacy, University of Toronto, Toronto, Ontario.

Annie Dufour, BSc, at the time of this study was a Pharmacy Student at the Faculty of Pharmacy, University of Toronto.

John Iazzetta, PharmD, is Coordinator, Drug Information, Department of Pharmacy, and Division of Clinical Pharmacology, Sunnybrook Health Science Centre and Associate Professor, Faculty of Pharmacy, University of Toronto.

Address correspondence to : Scott E. Walker, MScPhm, Department of Pharmacy, Sunnybrook Health Science Centre, 2075 Bayview Avenue, North York, Ontario M4N 3M5.

ambiante, que nous avons pu observer, le D5W représente le diluant de choix.

Mots clés : cloxacilline, coloration, relative aux concentrations, stabilité.

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INTRODUCTION

Information regarding the stability of cloxacillin in IV solutions is limited to 3 previous reports,[1-3] two[1,2] of which are primarily concerned with compatibility. Rank et al[1] reported only the tobramycin concentrations in a study evaluating the inactivation of tobramycin by penicillins. We have previously reported that cloxacillin precipitates immediately when mixed with hydromorphone solutions if the cloxacillin concentration exceeds 24 mg/mL when the diluent is 5% dextrose in water (D5W).[2] This study also indicated that more than 90% of the cloxacillin concentration remains after a 24-hour period in a solution containing 2 g cloxacillin diluted in 50 mL of D5W. Piwowarska et al[3] also reported the stability of cloxacillin, but only over a 24-hour period. In 1989, Travenol Canada and Beecham Laboratories completed a cloxacillin stability study using Beecham's Bactopen® product. The results of this study, as summarized in the 1990 product monograph printed in the CPS,[4] indicated that nominal concentrations between 0.5 mg/mL and 40 mg/mL in 0.9% sodium chloride (NS) or D5W could be stored under refrigeration for up to 14 days. However, by 1991, Bactopen® was withdrawn from the Canadian market and so this information is both difficult to locate and the original data remain unpublished.

The recommended dose of cloxacillin[5] ranges from 250 mg in 50 mL of an intravenous solution every 6 hours to a maximum of 6 g/day. Generally, this results in the preparation of concentrations not exceeding 20 mg/mL. More recently, outpatient administration of cloxacillin has resulted in doses of 12 g being diluted in 250 mL. In some outpatient programs it is common to dilute higher doses in sterile water to reduce the osmolarity to a more acceptable level.[6] Most patients can tolerate infusions of antibiotics into a peripheral vein as long as the osmolarity is less than 600 mOsm[6], but solutions of greater osmolarity may cause pain and vein irritation, especially when large volumes of hyperosmolar solutions are infused for prolonged periods. Conversely, hypoosmolar solutions may cause red cell hemolysis, but the degree of hemolysis is related to both the rate and volume of infusion as well as the osmolarity of the solution.

While stability information for cloxacillin has obviously been available, but unpublished, for concentrations less than 40 mg/mL, no data are available concerning the stability of higher concentrations. The primary objective of this study was to evaluate the stability of cloxacillin ranging in concentration from 5 to 50 mg/mL in different infusion solutions. Preliminary studies demonstrated that degradation of cloxacillin was accompanied by a change in pH and precipitation of cloxacillin from solution. Therefore, to better understand cloxacillin degradation, a secondary objective of the study was to evaluate the effect of pH on cloxacillin solubility and degradation rate.

METHODS

Assay Validation

Following the development of the chromatographic system for cloxacillin, the suitability of this method for use as a stability-indicating assay was tested by accelerating the degradation of cloxacillin. Cloxacillin (Cloxacillin Sodium for Injection - 2000 mg; Lot # : 118040; Novopharm Ltd, Toronto, ON) was dissolved in 50 mL of distilled water to prepare solutions of 40 mg/mL and 80 mg/mL. These solutions were incubated in a water bath at 88°C. Samples were chromatographed at time zero and every 30 minutes for 210 minutes. Chromatograms were inspected for the appearance of additional peaks and the cloxacillin peak was compared between samples for changes in concentration, retention time and peak shape.

Following assay development, the accuracy and reproducibility of standard curves was tested daily over 5 days and system suitability criteria (theoretical plates, tailing and retention time) were developed to ensure consistent chromatographic performance. On each day a known weight of approximately 200 mg of cloxacillin (Cloxacillin Sodium for Injection - 2000 mg; Lot # : 118040; Novopharm Ltd, Toronto, ON) was dissolved in 2 mL of water. This stock solution was then further diluted with water to obtain 9 standards with final concentrations of approximately 67, 60, 47, 40, 27, 20, 7, 3, and 2 mg/mL. When combined with a blank, these standards served to construct a standard curve. Five microlitres of each sample were chromatographed in duplicate. As well, 3 quality control samples of cloxacillin were prepared (known concentrations of approximately 53, 33, and 13 mg/mL) and chromatographed in duplicate each day. The determined concentrations were compared to their known concentrations. Intra-day and inter-day error was assessed by the coefficient of variation of the peak area of both quality control samples and standards.

Assay

Cloxacillin was quantified using a reverse phase liquid chromatographic system with UV detection at 250 nm.

The aqueous portion of the mobile phase was prepared by mixing 2 mL of triethylamine (TEA), 16.98 gm of tetrabutyl ammonium hydrogen sulphate (TBA) to 1 litre of distilled water. The pH of this solution was then adjusted to 6.0 with 1 M sodium hydroxide. The final mobile phase was prepared by mixing 35 parts of this TEA-TBA buffer with 65 parts of methanol. This mobile phase pumped (Model 510; Waters Scientific, Mississauga, ON) at 0.5 mL/min through a 15 cm x 4.6 mm, 3μm column (Supelcosil LC-18; Supleco, Bellefonte, PA). Injections were made with an automated injector (WISP 712, Waters Scientific, Mississauga, ON). Cloxacillin was detected at 250 nm using a variable wavelength detector (UV3000; Thermo Separation Products; San Jose, CA). Integration of the peak area at 250 nm was performed by a computer using chromatography acquisition and integration software (PC1000; Thermo Separation Products Inc., San Jose , CA). The cloxacillin peak area was subjected to least squares polynomial regression and the concentration, from the average of each sample, was interpolated from standard curves and recorded. Concentrations were recorded to the nearest 0.01mg/mL.

Effect of pH and Concentration on Degradation Rate

Known weights approximately 5 mg, 25 mg, 50 mg and 250 mg of cloxacillin (Cloxacillin Sodium for Injection - 2000 mg; Lot # : 118040; Novopharm Ltd, Toronto, ON) were dissolved in 25 mL of either 0.5 M KH_2PO_4 (pH of 4.5) or a mixture of 0.5 M K_2HPO_4 and 0.5 M KH_2PO_4 to achieve a final pH of 6.5. The pH of each solution was measured prior to each study and then each solution was incubated in a water bath at a known temperature (~77°C). Samples were drawn immediately prior to incubation and at 30-minute intervals for at least 210 minutes. Each sample was chromatographed and the cloxacillin concentration determined. At the end of each study, each solution was cooled to room temperature and the pH measured. A buffer strength of 0.5 M was based on the ability of the buffer to maintain the initial pH throughout the study period for all concentrations.

Effect of pH on Solubility

A known weight of approximately 4 g of cloxacillin (Cloxacillin Sodium for Injection - 2000 mg; Lot # : 118040; Novopharm Ltd, Toronto, ON) was dissolved in 25 mL of distilled water, NS or D5W. Known volumes of 0.1 N HCl were added to each solution while continuously monitoring the pH until the mixture had a pH less than 4.0. Following each addition of HCl, samples of known volume were taken for chromatography. Samples with precipitate were centrifuged and the supernate chromatographed. The concentration of cloxacillin and the pH immediately prior to the formation of a precipitate in each diluent (sterile water, NS, and D5W) were recorded.

Cloxacillin Solution Osmolarity

The osmolarity of 18 solutions having concentrations of 0, 5, 10, 20, 40, and 50 mg/mL in each of 3 diluents (D5W, NS, and sterile water) was tested by a freezing point depression method (Osmometer, Advance Instruments Inc., Boston MA) in the Biochemistry Department of our institution. One sample of each concentration and solution type was tested.

Stability Study

Due to the number of samples in this study, D5W solutions were prepared and studied and following completion of this analysis, NS solutions were prepared and then studied using the same sampling protocol. For each study, 7 vials of sterile cloxacillin powder (Cloxacillin Sodium for Injection - 2000 mg; Lot # : 118040; Novopharm Ltd, Toronto, ON) were each reconstituted with 6.8 mL of sterile water according to the manufacturer's instructions. This solution was diluted in sufficient D5W (Baxter Corporation, Toronto, ON) or NS (Baxter Corporation, Toronto, ON) to prepare 100 mL volumes of 5, 10, 20, 40, and 50 mg/mL. The 100 mL volume was divided into 8 samples of 10 mL of each concentration-solution type combination and placed in PVC containers. Four aliquots of each were stored at 4°C, and 4 were stored at room temperature (23°C). On study days 0, 1, 2, 4, 7, 8, 9, 10, 11, 14, 16, and 18 the cloxacillin concentration was determined by liquid chromatography. On each study day, fresh standards of cloxacillin were prepared and chromatographed as previously described, and the cloxacillin concentration from each of the samples was determined. Tests of physical inspection and pH were also completed and the observations recorded.

Data Reduction and Statistical Analysis

Means (± standard deviation) were calculated for replicated analyses. Reproducibility was assessed by coefficient of variation (CV). 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 using acid were compared for goodness of fit by the Maximum Likelihood Method of Box and Cox.[7,8] Analysis of variance with the least significant difference multiple range test (SPSS for Windows, Release 5.0.1, 1992) were used to compare differences between storage conditions (temperature, concentration and solution type). The 5% level was used as the *a priori* cut-off for significance.

In the stability portion of the study, cloxacillin 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. A solution was judged to be physically stable if there was no visual change in the colour or clarity of the mixture, there was no evidence of a precipitate or other particulate formation, and the concentration remained greater than 90% of the initial concentration.

RESULTS

Accelerated Degradation and Assay Validation

The stability-indicating liquid chromatographic method was validated using accepted stability indicating procedures.[9,10] Cloxacillin in distilled water at 88.0 ± 1.1°C degraded to less than 0.1% of the initial concentration within 210 minutes. Degradation appeared to occur in a first order fashion (first order r^2 for both concentrations > 0.9963; compared to zero order r^2 for both concentrations <0.6240), with a half-life of about 24 minutes. After 210 minutes, there was chromatographic evidence of degradation products. These degradation products eluted at different times than did cloxacillin and did not interfere with cloxacillin analysis. Based on the observation of predictable degradation and chromatographic separation of degradation products from cloxacillin, this analytical method was judged to be stability-indicating.[9,10]

Intra-day replication error averaged less than 1.7% for all standards. This was based on duplicate determinations over a 5-day validation period and the 27 standard curves prepared during the D5W and NS studies for all standards. Within-day accuracy, averaged for each standard over the 5-day validation period and the 27 standard curves prepared during the studies, ranged from -1.1% to 3.6%. The error of 3.6% was observed for the 2 mg/L standard solution. Reproducibility and accuracy for 3 quality control samples (53 mg/mL, 33 mg/mL and 13 mg/L) was similar, with replication error averaging less than 1.7% and accuracy within 3% for all 3 samples. These analyses indicated that the cloxacillin concentrations were measured accurately and reproducibly and that differences of 10% or more can be confidently detected with acceptable error rates.[11] 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.

Effect of pH and Concentration on Degradation Rate

Accurate known weights of approximately 5, 25, 50, and 250 mg of cloxacillin were dissolved in 25 mL of 0.5 M phosphate buffer with an initial pH of either 4.5 or 6.5. After 120 minutes of incubation at 77°C, approximately 36.74 ± 2.41% remained in solutions with an initial pH of 4.5 (concentrations of 0.2, 1, 2, and 10 mg/mL) while 28.01 ± 2.48 remained in solutions with an initial pH of 6.5. The degradation rate appeared to be first order and the apparent half-life averaged 79.7 ± 3.2 minutes at pH 4.5 and 66.9 ± 2.1 minutes at pH 6.5. The difference due to pH was significant (p=0.014) while concentration had no effect (p=0.925) on the degradation rate.

Effect of pH on Solubility

The solubility of sodium cloxacillin in aqueous solutions was observed to be pH dependent. At a pH greater than 4.2, the solubility of fresh sodium cloxacillin in aqueous solutions of water, NS or D5W exceeds 100 mg/mL. Below this pH, the solubility is extremely sensitive to pH and drops to 89.03 mg/mL at pH 4.2 and again to 63 mg/mL at a pH of 3.5. The solubility was not dramatically affected by other solutes in the water such as dextrose or sodium chloride, as the solubility in sterile water, D5W, and NS was similar.

Solution Osmolality

The osmolarity observed for 18 solutions ranging in concentration from 0 to 50 mg/mL in each of 3 diluents (D5W, NS, and sterile water) is listed in Table I. Solutions of cloxacillin diluted in sterile water have osmolarities of 204 or less even when the concentration of cloxacillin is as great as 50 mg/mL. These solutions were considered to be too hypo-osmolar for use as continuous infusions. However, solutions of cloxacillin from 5 mg/mL to 50 mg/mL in either NS or D5W have osmolarities between 300 and 432 mOsm. Solutions with osmolarity of this strength are ideal for intravenous use.

Table I. Osmolality of Cloxacillin Solutions.

Cloxacillin Concentration (mg/mL)	Sterile Water (mOsm)	Normal Saline (mOsm)	5% Dextrose in Water (mOsm)
0	1	286	258
5	18	307	285
10	45	324	301
20	90	359	332
40	163	408	388
50	204	432	415

Stability Study

At room temperature, all NS solutions retained more than 90% of their initial concentration for only 24 hours and linear regression indicated that all concentrations, except the 5 mg/mL solution, had lost more than 10% of their initial concentration within 48 hours. These solutions developed a precipitate between the third day of storage (40 and 50 mg/mL solutions) and the eighth day of storage (5 mg/mL solution - Table II). The pH of these solutions was initially between 5.6 and 5.9 (Table II) and each solution declined by between 1.40 and 1.58 pH units by day 4. Thereafter, the pH changed by less than 0.2 of a pH unit.

D5W solutions were more stable ($p<0.001$). Ninety percent of the initial concentration was retained for up to 4 days at all concentrations except the 50 mg/mL solution. Linear regression indicated that the time to achieve 90% of the initial concentration was extended from 1.73 days at 50 mg/mL to 3.77 days at 5 mg/mL (Table II). The initial pH of these solutions was slightly lower than NS solutions, varying with concentration from 4.94 to 5.32 (Table II). The pH of these solutions declined by between 0.71 and 0.88 pH units by day 8. Thereafter, the pH changed by less than 0.2 of a pH unit. After 18 days at room temperature, D5W solutions had between 33.54% (50 mg/mL) and 47.9% (5 mg/mL) remaining, whereas the cloxacillin concentration of NS solutions ranged from 28.37% (50 mg/mL) to 38.5% (5 mg/mL) remaining.

Under refrigeration, D5W and NS solutions both retained more than 90% of the initial concentration for 18 days but showed chromatographic evidence of a small degree of degradation. NS solutions had an initial pH of between 5.59 and 5.87. The pH declined by 1 pH unit in all solutions by day 18. The pH of D5W solutions was initially slightly lower than saline solutions, ranging between 4.93 and 5.77. Over the 18-day study period, the drop in pH averaged 0.73 pH units.

While the rate of degradation appears related to concentration, it may in fact be driven by pH. For both solutions at both temperatures, the initial pH of cloxacillin concentrations was higher. Non-linear (second order polynomial) regression of the hydrogen ion concentration vs. degradation rate or the time to achieve 90% of the initial concentration, yields a significant relationship ($r^2=0.9079$; $p<0.001$).

Table II. Summary of Cloxacillin Concentrations and pH

	Nominal Initial Cloxacillin Concentration				
	5 mg/mL	10 mg/mL	20 mg/mL	40 mg/mL	50 mg/mL
D5W Solutions stored at 4°C					
Initial concentration (mg/mL ± Std Dev)	4.4 ± 0.04	8.7 ± 0.1	17.7 ± 0.2	37.4 ± 0.3	44.8 ± 0.3
Days greater that 90% Remaining	18	18	18	18	18
Percent Remaining on Day 18 [δ]	98.9	96.2	93.7	93.4	97.1
Regression predicted T-90 (days) [δ]	167.69	47.63	28.35	27.17	62.12
Time to visual precipitate (days) [Φ]	none	none	none	none	none
Initial pH, day 0	4.93	5.40	5.51	5.65	5.77
pH Day 18	4.62	4.61	4.71	4.80	4.88
Normal Saline Solutions stored at 4°C					
Initial concentration (mg/mL ± Std Dev)	4.5 ± 0.7	8.7 ± 0.4	18.5 ± 0.5	36.8 ± 2.9	43.1 ± 1.1
Days greater that 90% Remaining	18	18	18	18	18
Percent Remaining on Day 18 [δ]	102.8	101.9	94.4	97.4	93.1
Regression predicted T-90 (days) [δ]	69.97	113.06	33.08	68.78	26.83
Time to visual precipitate (days) [Φ]	none	none	none	none	none
Initial pH, day 0	5.66	5.59	5.65	5.79	5.87
pH Day 18	4.89	4.60	4.58	4.69	4.78
D5W Solutions stored at 23°C					
Initial concentration (mg/mL ± Std Dev)	4.4 ± 0.1	9.0 ± 0.1	18.0 ± 0.2	36.8 ± 0.3	44.9 ± 0.3
Days greater that 90% Remaining	4	4	4	4	2
Percent Remaining on Day 18 [δ]	47.92	39.75	38.50	37.46	33.54
Regression predicted T-90 (days) [δ]	3.77	3.01	2.59	1.93	1.73
Time to visual precipitate (days) [Φ]	7	4	4	4	4
Initial pH, day 0	4.94	5.04	5.14	5.24	5.32
pH Day 18	4.20	4.21	4.43	4.71	4.78
Normal Saline Solutions stored at 23°C					
Initial concentration (mg/mL ± Std Dev)	4.7 ± 0.0	8.5 ± 0.4	19.0 ± 0.5	36.4 ± 0.9	44.8 ± 1.1
Days greater that 90% Remaining	1	1	1	1	1
Percent Remaining on Day 18 [δ]	38.52	35.05	38.08	27.53	28.37
Regression predicted T-90 (days) [δ]	2.10	1.92	1.98	1.53	1.50
Time to visual precipitate (days) [Φ]	8	4	4	3	3
Initial pH, day 0	5.66	5.59	5.65	5.79	5.87
pH Day 18	4.06	4.08	4.30	4.55	4.64

[δ] Time (days) for the cloxacillin concentration to change by 10%. Concentrations remaining are based on least squares linear regression of concentration time data for each temperature-solution-concentration combination.

[Φ] Time in days for a precipitate to appear in the solution or for an initially clear and colourless solution became cloudy.

DISCUSSION

Cloxacillin remains a highly useful antibiotic and is now being used by many institutions for outpatient therapy. We have demonstrated that NS or D5W should be used as the diluents for

cloxacillin solutions as the osmolarity is optimal. It can be predicted from the osmolarity results that if half-normal saline was used as the diluent, the cloxacillin concentration would have to exceed 40 mg/mL before the osmolarity would rise above 250 mOsm. Therefore, both sterile water and half-normal saline should not be considered suitable diluents for cloxacillin solutions that are to be used for peripheral intravenous infusion.

This study has also demonstrated that the stability of commercially available cloxacillin, diluted in NS or D5W, is concentration, solution and temperature dependent. The concentration dependency is most evident at room temperature in D5W solutions, where the 40 and 50 mg/mL retain only 90% of their initial concentration for 24 hours while the 5 mg/mL solution retains 90% of the initial concentration for 72 hours. It is also apparent that the degradation rate is greater in NS than D5W solutions and for solutions stored at room temperature compared to those stored in the refrigerator. However, while the degradation rate appears to be related to concentration, it may be driven by pH or the hydrogen ion concentration. As was observed in the study evaluating the effect of pH and concentration (completed at ~77°C), the degradation rate was faster at a pH of 6.5 compared to 4.5. Since the NS solutions had a higher initial pH than the D5W solutions and since the more concentrated solutions also had a higher pH than the more dilute solutions, the faster degradation rates observed for both the more concentrated solutions and for the saline solutions would appear to be related to the initial pH of the solution. Furthermore, while precipitation also appears to be concentration dependent, in a previous paper[2] precipitation was ascribed to the presence of citric acid and it might be inferred that precipitation was also pH dependent. We have confirmed that cloxacillin solubility is pH dependent. However, the observed limit of solubility at a pH of 4.2 was approximately 100 mg/mL, and this exceeded the highest concentrations evaluated in this study. Nevertheless, the 50 mg/mL solution precipitated after 4 days of storage when the pH measured 4.5. Clearly, while pH may be a factor which causes cloxacillin to precipitate, another factor, possibly the concentration of cloxacillin degradation products, may also affect cloxacillin solubility.

No previous report has evaluated cloxacillin stability beyond 24 hours. The only cloxacillin stability study which is similar to our report was completed in 1989 by Travenol Canada and Beecham Laboratories using Beecham's Bactopen® product. In this 21-day study, the 40 mg/mL (2000 mg/50 mL polyvinyl chloride bag of NS or D5W) and 25 mg/mL (1250 mg/50 mL PVC bag of NS or D5W) concentrations achieved 95% of the initial concentration on day 14.* Our results at 4°C are similar in that, while there is some concentration dependency to degradation, about 95% of the initial concentration remained on day 18.

A recommended expiry date must consider that a prepared product may be stored for a period of time at both 4°C and room temperature. Based on the faster rate of degradation observed at room temperature for NS solutions, D5W is the preferred diluent. In studies of cloxacillin diluted in D5W and stored at room temperature, it was observed that approximately 95% of the initial cloxacillin solution remained after 1 day. Furthermore, all solutions stored at 4°C retained more than 95% of the initial concentration for 14 to 18 days. Therefore, it is estimated that solutions stored for 14 days at 4°C and then allowed to stand for no more than 24 hours at room temperature would contain more than 90% of the initial concentration. Additional storage at room temperature of higher concentrations could result in the cloxacillin concentration falling below 90% of the initial concentration. Furthermore, concern for the possibility of beta-lactam degradation products potentially increasing antibody titres, as was observed with penicillin degradation products,[12] and knowing that extending an expiry date beyond 7 days may not further reduce wastage of a frequently prescribed antibiotic[13,14] has persuaded us to recommend that cloxacillin diluted in D5W be stored at 4°C for up to 7 days may be prudent. These solutions could then be allowed to stand for no more than 24 hours at room temperature and would still maintain almost 95% of the initial concentration.

* John Parks, Regulatory Affairs, Baxter Corporation. Personal communication October 21, 1997 concerning data which are unpublished but on file at Baxter Corporation. Study completed on Bactopen® (Beecham Laboratories Inc.) using products that were within 6 and 12 months of shelf life expiration.

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