

# Stability of Trisodium Citrate and Gentamicin Solution for Catheter Locks after Storage in Plastic Syringes at Room Temperature

Dennis Cote, Charmaine E Lok, Marisa Battistella, and Lavern Vercaigne

## ABSTRACT

**Background:** Catheter-related infections are a major problem for hemodialysis patients with central venous catheters for vascular access. Catheter lock solutions containing an anticoagulant are used to maintain the patency of the catheter between hemodialysis sessions. There is evidence that the use of lock solutions containing an antibiotic is associated with lower rates of infection but also that these solutions can kill microbes in colonized catheters and thus avoid the risks and costs associated with replacing the catheter.

**Objective:** This stability study was conducted to determine whether an extemporaneously prepared gentamicin–citrate catheter lock solution would retain its potency over time, thus allowing for advance preparation of the solution.

**Methods:** Catheter lock solutions containing gentamicin alone, citrate alone, and the combination of gentamicin and citrate were prepared aseptically and packaged in polyethylene syringes. The syringes were stored at room temperature. At timed intervals over 112 days, samples were withdrawn for analysis by means of validated high-performance liquid chromatography.

**Results:** None of the 3 lock solutions showed any evidence of degradation during the 112-day observation period. In the formulation containing both gentamicin 2.5 mg/mL and sodium citrate 40 mg/mL (4%), there was no change in the concentration of either gentamicin ( $p = 0.34$ ) or citrate ( $p = 0.55$ ). Linear regression analysis of the concentration–time data for the combined formulation showed that 99.97% of the labelled amount of gentamicin and 101.30% of the labelled amount of citrate remained at day 112. The lower limit of the 95% confidence intervals indicated that more than 98.17% of the gentamicin and more than 99.57% of the citrate remained on day 112.

**Conclusion:** The results of this study will allow pharmacies to extemporaneously compound the combined gentamicin–citrate catheter lock solution in advance of use. The method described here will yield a stable product for use in clinical applications.

**Key words:** gentamicin, citrate, stability, catheter lock solution, hemodialysis, high-performance liquid chromatography

## RÉSUMÉ

**Contexte :** Les infections liées aux cathéters sont un problème important chez les patients hémodialysés dont l'abord vasculaire est réalisé à l'aide de cathéters veineux centraux. On a recours à des solutions pour verrou de cathéter contenant un anticoagulant afin de maintenir la perméabilité du cathéter entre les séances d'hémodialyse. Des données montrent que des solutions pour verrou renfermant un antibiotique sont associées à des taux d'infections inférieurs et que ces solutions peuvent tuer les microbes colonisant les cathéters, ce qui permet donc d'éviter les risques et les coûts associés au remplacement des cathéters.

**Objectif :** Cette étude de stabilité a été menée pour déterminer si une solution extemporanée de gentamicine et de citrate pour verrou de cathéter pouvait conserver sa puissance dans le temps, ce qui permettrait ainsi de préparer la solution à l'avance.

**Méthodes :** Des solutions pour verrou de cathéter contenant de la gentamicine seule, du citrate seul et l'association gentamicine-citrate ont été préparées selon une méthode aseptique, puis conditionnées dans des seringues de polyéthylène. Les seringues ont été conservées à la température ambiante. À différents points dans le temps sur une période de 112 jours, des échantillons ont été prélevés à des fins d'analyse à l'aide d'une épreuve validée par chromatographie liquide haute performance.

**Résultats :** Aucune des trois solutions pour verrou n'a montré de signes de dégradation durant les 112 jours de la période d'observation. La préparation contenant la gentamicine à 2,5 mg/mL et le citrate de sodium à 40 mg/mL (4 %) n'a montré aucun changement dans la concentration de gentamicine ( $p = 0,34$ ) et de citrate ( $p = 0,55$ ). Une analyse de régression linéaire des concentrations en fonction du temps de la préparation associant la gentamicine et le citrate a révélé que cette préparation avait retenu 99,97 % de sa concentration initiale de gentamicine et 101,30 % de sa concentration initiale de citrate au jour 112. La limite inférieure de l'intervalle de confiance à 95% a également révélé que la préparation avait retenu plus de 98,17 % de la gentamicine et plus de 99,57 % du citrate au jour 112.

**Conclusion :** Les résultats de cette étude permettront aux pharmacies de préparer à l'avance la solution extemporanée pour verrou de cathéter renfermant l'association gentamicine–citrate. La méthode décrite ici donnera un produit stable pour une utilisation dans des applications cliniques.

**Mots clés :** gentamicine, citrate, stabilité, solution pour verrou de cathéter, hémodialyse, chromatographie liquide haute performance

[Traduction par l'éditeur]

## INTRODUCTION

Catheter-related infections are a major problem for hemodialysis patients with central venous catheters for vascular access. Bacteremia may result from microorganisms entering the bloodstream at the catheter insertion site or from contamination of the catheter lumen.<sup>1</sup> Various strategies may be used to prevent this complication, including aseptic precautions when manipulating or accessing the catheter and scrupulous hygiene of the skin around the catheter exit site.<sup>2</sup>

The formation of a bacterial biofilm on the wall of the catheter lumen has been implicated in catheter colonization.<sup>3</sup> In most cases of catheter-related bacteremia, treatment with systemic antibiotics alone is insufficient to resolve the infection, because the biofilm provides a nidus for infection and recurrent bacteremia. The affected catheter must usually be removed, but in selected cases a lock solution containing both an antibiotic and an anticoagulant is instilled into the catheter lumen. This approach results in a cure in about two-thirds of cases of catheter-related bacteremia, thus avoiding the risks and costs associated with replacing the catheter.<sup>2</sup> Lock solutions are used to maintain the patency of the catheter between hemodialysis sessions, and several recent publications have supported the use of antibiotic–anticoagulant lock solutions to reduce the rate of catheter-related infection.<sup>4–8</sup>

Heparin is an anticoagulant that is commonly used for maintaining catheter patency, but citrate has also been shown to be effective in this respect; furthermore, at concentrations of about 30%, citrate has shown some antimicrobial activity.<sup>9</sup> Although locking solutions containing a high concentration of citrate are effective, they are not used in North America, because of concerns about patient safety, particularly the possibility of cardiac arrest.<sup>10</sup> Investigations of lower-concentration citrate lock solutions demonstrated that a solution containing 4% trisodium citrate (40 mg/mL) was both effective and safe.<sup>11,12</sup>

A number of antibiotics, including vancomycin and gentamicin, have been used in lock solutions.<sup>13</sup> At present, gentamicin appears to be acceptable for this purpose, and it has been used in combination with citrate as a lock solution. With gentamicin-containing solutions, catheter-related infection rates decreased from about 4 episodes per 1000 catheter days to 0.3 episodes per 1000 catheter days.<sup>14,15</sup>

A mixture of gentamicin and citrate suitable for use as a locking solution is not available commercially, so it must be compounded from commercially available components. Efficiencies in terms of time and cost can be achieved by preparing and storing such solutions in advance of their use, but information regarding the product's stability is required.<sup>16,17</sup> In one stability study, in which commercial 46.7% trisodium citrate solution was used to extemporaneously prepare a 4%

solution, there was no change in concentration after 28 days of storage in plastic syringes at room temperature.<sup>18</sup> Although the compatibility of a gentamicin–citrate solution has been studied,<sup>19</sup> the findings were of limited value since the investigators only examined the solutions for visual changes over time; they did not measure the concentrations of the components.

This type of admixture prepared in quantity would be considered to have a medium risk of microbial contamination. USP Chapter <797> would assign an expiration period of 30 h at room temperature and 7 days with refrigeration.<sup>16</sup> However, expiration periods can be expanded if published data showing compatibility and stability of the product are available and sterility testing is performed.<sup>17</sup> Therefore, the purpose of this stability study was to determine whether an extemporaneous preparation of a gentamicin–citrate catheter lock solution packaged in prefilled syringes retained its potency over a reasonable period of time, to allow for advance preparation and storage of the solution.

## METHODS

### Chemicals, Reagents, and Materials

The study was performed between November 2007 and May 2008. Commercially prepared gentamicin 40 mg/mL for injection (Sandoz Canada Inc, Boucherville, Quebec; lot 139819, expiry May 2009), sodium citrate 46.7% for injection (TriCitrasol, Cytasol Laboratories Inc, Braintree, Massachusetts; lot B82T, expiry January 2011), and USP-grade sterile water for injection (Hospira Health Care Corp, Montréal, Quebec; lot 74-337-DK, expiry January 2011) were used to prepare the study solutions, which were stored in polyethylene syringes with luer-lock caps (MedXL Inc, Montréal, Quebec; lot F86331). Gentamicin sulphate USP (PCCA Canada, London, Ontario; lot C104339) and sodium citrate USP (Spectrum, Gardena, California; lot KC066) were used as reference standards. High-performance liquid chromatography (HPLC)–grade acetonitrile (lot 891499) and phosphoric acid (lot 082037) and analytical-grade hydrochloric acid (lot 4100030) and sodium hydroxide (lot 016045) were purchased from Fisher Scientific (Nepean, Ontario). Hydrogen peroxide USP (6%) was purchased from Pure Standard Products (Edmonton, Alberta; lot 189). Phenylisocyanate (Fluka; lot 1378398), triethylamine (lot 047K1041), and trifluoroacetic acid (Fluka; lot 046710) were purchased from Sigma-Aldrich (St Louis, Missouri). Samples for analysis were stored frozen in cryovials (Nunc, Rochester, New York; lot 089675), and the mobile phase was filtered through Supor-200 polyethersulfone 0.2- $\mu$ m membranes (Pall, Gelman Laboratories, East Hills, New York; lot 51321).

## HPLC Assays

The chromatographic system was manufactured by Knauer (Berlin, Germany) and consisted of a Wellchrom K-501 pump, a Wellchrom K-2501 variable-wavelength ultraviolet (UV) detector, a Basic-Marathon autosampler, and a Wellchrom HPLC interface box. Eurochrome 2000 operating and data-acquisition software (Knauer, Berlin, Germany) was used. All chromatography was performed at room temperature, and the temperature of the experimental environment was recorded periodically over the study period.

For the gentamicin analysis, an isocratic reversed-phase HPLC method based on that of Kim and others<sup>20</sup> was followed, except for use of a 150 × 4.6 mm cyano column with a particle size of 4 µm (Jones, Hengoed, UK; lot 9650001) instead of a C18 column. The mobile phase consisted of acetonitrile–water–trifluoroacetic acid (400:600:1 v/v/v) at a flow rate of 1.0 mL/min. The injection volume was 20 µL, detection was by UV absorbance at 240 nm, and the concentration of gentamicin was quantified by means of external standards and peak areas. A stock solution containing gentamicin 400 µg/mL in water was made fresh daily and was used to prepare the standard solutions, also in water. Because gentamicin has no inherent UV-absorbing properties, phenylisocyanate derivatives of the drug, which absorb strongly at 240 nm, were prepared according to the method described by Kim and others.<sup>20,21</sup> Briefly, each sample was diluted with water to a gentamicin concentration of about 250 µg/mL. A 0.5-mL volume of this solution was then mixed with 0.25 mL of a 0.5% v/v solution of triethylamine in acetonitrile, and 0.25 mL of a 0.5% v/v solution of phenylisocyanate in acetonitrile was added. Kim and others<sup>20</sup> reported that the reaction is very rapid at room temperature and the derivatives are stable for at least 24 h at room temperature. The phenylisocyanate and triethylamine reagents are also stable at room temperature for at least 24 h.<sup>21</sup>

For citrate analysis, an isocratic ion-exchange HPLC method, as described in a Supelco application note,<sup>22</sup> was used. The mobile phase consisted of 0.1 % w/v phosphoric acid at a flow rate of 0.5 mL/min. A 300 × 7.8 mm polystyrene divinylbenzene resin column (Supelcogel C-610H, Supelco Canada, Mississauga, Ontario; lot 59320U) was used. The injection volume was 20 µL, detection was by UV absorbance at 210 nm, and the concentration of citrate was quantified with external standards. A stock solution containing sodium citrate 3200 µg/mL in water was made fresh daily and was used to prepare the standard solutions. Samples were prepared by diluting the test solution with water to a concentration of sodium citrate of about 1600 µg/mL.

A typical run for analysis of the stored samples was 14 samples, 1 blank, and 6 standards. For gentamicin, the standards consisted of 2 each at 160, 240, and 320 µg/mL, representing about 65%, 100%, and 130% of the target

concentration; for citrate the standards consisted of 2 each at 800, 1600, and 2400 µg/mL, representing about 50%, 100%, and 150% of the target concentration. The samples were diluted in water to 250 µg/mL for gentamicin (1:10) and to 1600 µg/mL for citrate (1:25). The resulting 21 vials were coded and randomized, phenylisocyanate derivatives were prepared in the case of the gentamicin samples, and the samples were run. Quantitation was achieved by regression analysis of the standards, with the slope and intercept coefficients being used to calculate the concentrations of the samples from their respective peak areas.

## Validation of Gentamicin Assay

Three solutions of gentamicin standard (40 mg dissolved and made up to 100 mL in water) were prepared. These solutions were further diluted with water to prepare 3 sets of gentamicin solutions with concentrations of 80, 160, 240, 320, and 400 µg/mL. Phenylisocyanate derivatives were prepared from samples of each of these solutions, and the resulting solutions were analyzed as described above. These data were used to calculate the linearity and range of the method, with linear regression of response or peak area as a function of concentration.

For gentamicin, 240 µg/mL was selected as the target concentration, and the solutions at 160, 240, and 320 µg/mL, representing about 66%, 100%, and 130% of the target concentration, were used to determine accuracy and precision. The coefficients from the previous regression analysis were used to calculate a concentration value for each standard solution, which were expressed as a percentage (calculated concentration divided by theoretical concentration). Accuracy was assessed from the mean of the 9 values and precision from the relative standard deviation (SD) of this mean. On the following day, 3 fresh solutions were prepared and diluted to generate another 3 solutions with concentrations of 160, 240, and 320 µg/mL. Phenylisocyanate derivatives were prepared and analyzed. The resulting data and the data from the previous day were used to calculate between-day precision.

Forced degradation was used to demonstrate the specificity of the method. One-millilitre volumes of each of 6% H<sub>2</sub>O<sub>2</sub>, 0.1 mmol/L HCl, and 0.1 mmol/L NaOH were mixed individually with 1.0 mL of gentamicin 40 mg/mL in 100-mL flasks and left overnight. A control flask, containing only the gentamicin solution, was also prepared. The next morning, each flask was made up to volume with water, and four 0.5-mL samples were taken from each flask for preparation of phenylisocyanate derivatives and HPLC analysis.

To determine whether the presence of citrate would interfere with analysis of gentamicin, samples containing gentamicin 2.5 mg/mL and sodium citrate 40 mg/mL were also prepared. For this part of the study, 4 solutions were prepared:

gentamicin alone, citrate alone, gentamicin and citrate combined, and a control (distilled water). Phenylisocyanate derivatives were prepared from 4 samples from each flask, and these solutions were analyzed for gentamicin content.

## Validation of Citrate Assay

Three solutions of sodium citrate standard (320 mg dissolved and made up to 100 mL in water) were prepared. These solutions were further diluted with water to prepare 3 sets of citrate solutions with concentrations of 400, 800, 1600, 2400, and 3200 µg/mL. Samples from each of these solutions were analyzed using the HPLC assay described above. These data were used to calculate the linearity and range of the method, with linear regression of response or peak area as a function of concentration.

For sodium citrate, 1600 µg/mL was selected as the target concentration, and the solutions at 800, 1600, and 2400 µg/mL, representing about 50%, 100%, and 150% of the target concentration, were used to determine accuracy and precision. The coefficients from the previous regression analysis were used to calculate a concentration value for each standard solution, which were expressed as a percentage (calculated concentration divided by theoretical concentration). Accuracy was assessed from the mean of the 9 values and precision from the relative SD of this mean. On the following day, 3 fresh solutions were prepared and diluted to generate another 3 solutions with concentrations of 800, 1600, and 2400 µg/mL. Samples of these solutions were analyzed. The resulting data and the data from the previous day were used to calculate between-day precision.

Forced degradation was used to demonstrate the specificity of the method. One-millilitre volumes of each of 6% H<sub>2</sub>O<sub>2</sub>, 1 mmol/L HCl, and 1 mmol/L NaOH were mixed individually with 0.342 mL of sodium citrate 46.7% in 100-mL flasks and left overnight. Two controls, one consisting of 0.342 mL of citrate and one containing only 1.0 mL of H<sub>2</sub>O<sub>2</sub>, were also prepared. The next morning, each flask was made up to volume with water, and four 0.1-mL samples were taken from each flask for HPLC analysis.

To determine whether the presence of gentamicin would interfere with analysis of citrate, samples containing sodium citrate 40 mg/mL and gentamicin 2.5 mg/mL were also prepared. For this part of the study, 4 samples were prepared; citrate alone, gentamicin alone, citrate and gentamicin combined, and a control (distilled water). Four samples from each flask were analyzed for citrate content.

## Protocol for Stability Study

Three formulations were evaluated for compatibility and stability. These were prepared, using aseptic technique, from

the commercially available gentamicin (40 mg/mL) and trisodium citrate (46.7% for injection, diluted with water). Formulation A consisted of gentamicin 2.5 mg/mL, formulation B consisted of sodium citrate 40 mg/mL, and formulation C consisted of both gentamicin and sodium citrate at concentrations of 2.5 and 40 mg/mL, respectively. A 30-mL volume of each formulation was prepared, and 5-mL aliquots of each formulation were packaged into six 5-mL polyethylene syringes. The syringes were closed with luer-lock syringe tips and were stored at room temperature in a plastic bin with an opaque, snug-fitting lid.

On days 0, 7, 14, 28, 42, 56, and 112, a sample of about 0.8 mL was withdrawn from each of 4 randomly selected syringes for each formulation. These samples were placed in 1.8-mL screw-top cryovials and stored at -20°C until analysis. Both gentamicin and citrate have been shown to be stable for 6 months when frozen and stored at -20°C.<sup>23,24</sup> For gentamicin analysis, the samples were diluted 1:1 with water to give a nominal concentration of 250 g/mL; phenylisocyanate derivatives were prepared from 0.5-mL volumes of these solutions, as previously described, and analyzed by means of HPLC. For citrate analysis, samples were diluted 1:25 with water to generate a nominal concentration of 1600 µg/mL, and these solutions were analyzed by HPLC. The concentration of gentamicin and citrate was quantified with reference to standards analyzed concurrently with the samples. At the time of analysis, all samples, standards, and a blank were coded and analyzed in random order.

At the start and finish of the study, the pH of the formulations was assessed using a pH meter (Accumet Basic AB15, Fisher Scientific, Nepean, Ontario). Colour and clarity were assessed visually.

## Statistical Analysis

Statistical analyses were performed with SigmaStat (SPSS Inc, Chicago, Illinois) and Excel 2000 (Microsoft) software. All statistical analyses were performed at the 95% significance level, and data are presented as means ± SD, unless indicated otherwise. Linear regression models were constructed to estimate the amount, with 95% confidence intervals (CIs), of gentamicin and citrate remaining at the end of the 112-day observation period. In each model, the independent variable was time, and the dependent variables were the concentrations of gentamicin and citrate. Analysis of variance (ANOVA) was used to test changes in concentration over time.

## RESULTS

### Validation of Gentamicin Assay

Regression analysis of peak area as a function of concentration for standard solutions of gentamicin with concentra-

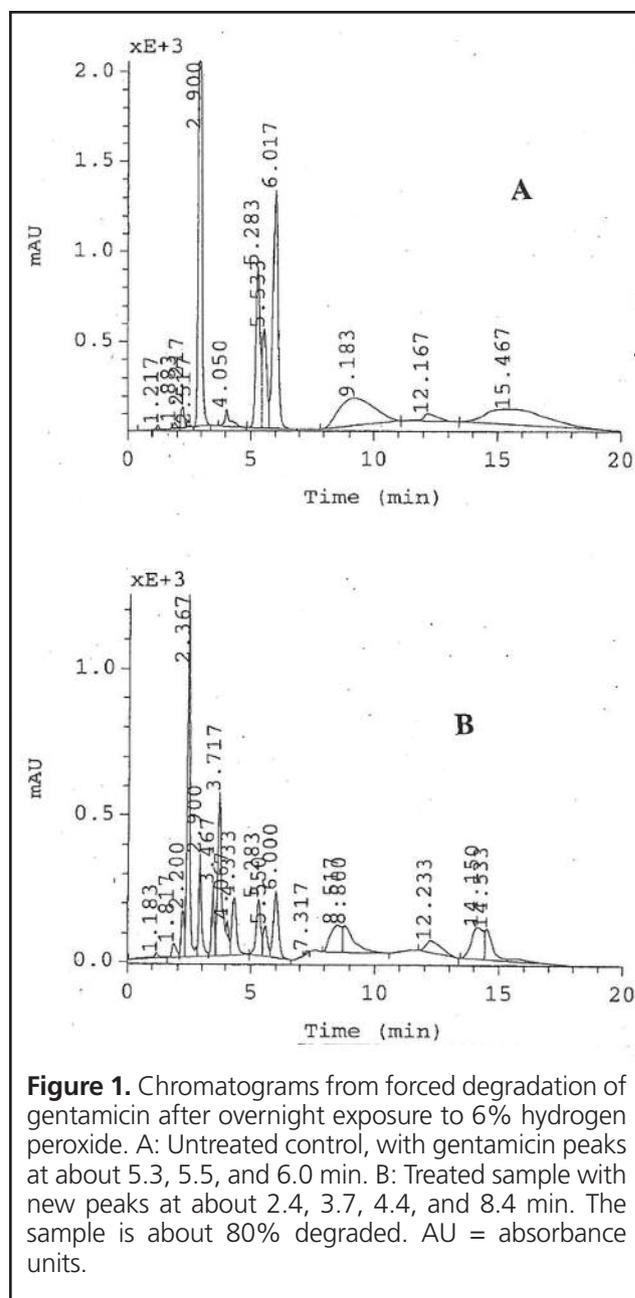
tions ranging from 80 to 400 µg/mL demonstrated linearity, and the coefficient of determination ( $r^2$ ) was 0.9924 ( $n = 15$ ). The 3 sets of solutions at concentrations of 160, 240, and 320 µg/mL had an accuracy of 101.0% ( $n = 9$ ) and a within-day precision, expressed as relative SD, of 2.21% ( $n = 9$ ). The between-day precision was 2.52% ( $n = 18$ ). Chromatograms of the phenylisocyanate derivatives prepared from the gentamicin standard showed 3 peaks eluting at 5.3, 5.6, and 6.0 min, which represented gentamicin  $C_1$ ,  $C_{1A}$ , and  $C_2$ .<sup>20</sup> To quantify the gentamicin, the areas of these 3 peaks were summed. Gentamicin  $C_2$  and  $C_{2A}$  are isomers that this assay was unable to resolve; they co-eluted as one peak, so the chromatograms showed 3 rather than 4 peaks. Other authors have quantified gentamicin on the basis of the sum of 3 peaks.<sup>25,26</sup>

The specificity of the method was established using forced degradation. The samples mixed with 0.1 mmol/L HCl showed no indication of degradation, and the gentamicin concentration remained essentially unchanged, with 99.63% ± 0.29% remaining for the HCl-treated samples and 99.10% ± 0.57% remaining for the control. The samples treated with 0.1 mmol/L NaOH showed some degradation, with 98.21% ± 0.29% remaining and the presence of a new peak at 4.4 min. The samples mixed with  $H_2O_2$  also showed signs of degradation, with 4 new peaks appearing on the chromatogram. Quantification of the gentamicin in these samples indicated that only 18.11% ± 0.83% of the drug originally present remained. Three of the new peaks eluted early, with retention times of 2.4, 3.7, and 4.4 min, respectively, and the fourth eluted late, at about 8.4 min. The peaks associated with gentamicin eluted between 5 and 6 min. Chromatograms of the control and peroxide-treated samples are presented in Figure 1.

Samples containing gentamicin alone, citrate alone, gentamicin and citrate combined, and a control (distilled water) were analyzed to determine whether the presence of citrate in samples would interfere with analysis of gentamicin. The 2 solutions containing gentamicin yielded similar chromatograms, with no statistically significant differences between them in terms of peak area ( $p = 0.92$ ). The sample containing only citrate generated no peaks and was identical with the chromatogram of the control preparation. These results indicated that the presence of citrate in the sample did not affect the analysis of gentamicin.

### Validation of Citrate Assay

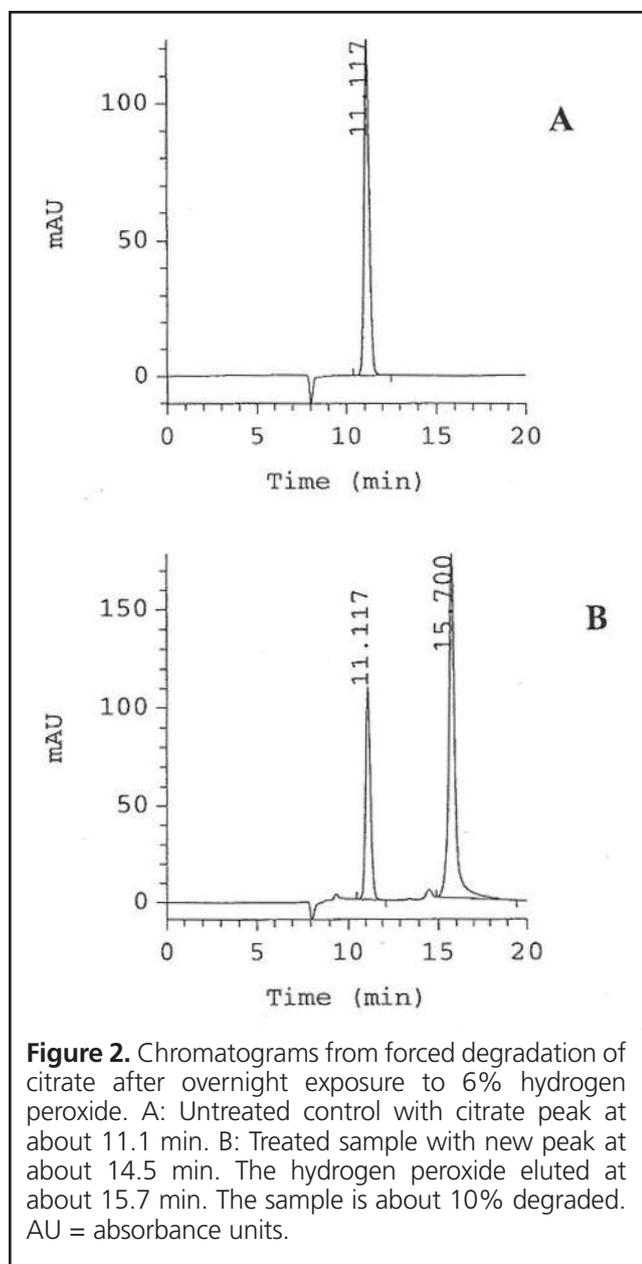
Regression analysis of peak area as a function of concentration for standard solutions of sodium citrate with concentrations ranging from 400 to 3200 µg/mL demonstrated linearity, and the coefficient of determination ( $r^2$ ) was 0.9996 ( $n = 15$ ). The 3 sets of solutions at concentrations 800, 1600, and 2400 µg/mL had an accuracy of 99.61% ( $n = 9$ ) and a within-day precision, expressed as relative SD, of 1.06% ( $n = 9$ ). The



**Figure 1.** Chromatograms from forced degradation of gentamicin after overnight exposure to 6% hydrogen peroxide. A: Untreated control, with gentamicin peaks at about 5.3, 5.5, and 6.0 min. B: Treated sample with new peaks at about 2.4, 3.7, 4.4, and 8.4 min. The sample is about 80% degraded. AU = absorbance units.

between-day precision was 0.88% ( $n = 18$ ). Chromatograms of citrate showed one peak eluting at 11.0 min.

Forced degradation was used to demonstrate the specificity of the method. The samples mixed with either 1 mmol/L HCl or 1 mmol/L NaOH showed no indication of degradation, and the citrate concentration remained essentially unchanged, with 99.92% ± 0.08% remaining for the HCl-treated samples, 99.97% ± 0.14% remaining for the NaOH-treated samples, and 100.0% ± 0.39% remaining for the control. The sample mixed with  $H_2O_2$  did show signs of degradation. The content remaining dropped to 91.98% ± 0.21% and a new peak appeared at 14.5 min, but it was too small to be integrated. The



peak for citrate had a retention time of 11.1 min, and the peak for  $H_2O_2$  had a retention time of 15.7 min. Chromatograms of the control and peroxide-treated samples are presented in Figure 2.

Samples containing citrate alone, gentamicin alone, both citrate and gentamicin, and a control (distilled water) were analyzed to determine whether the presence of gentamicin in samples would interfere with analysis of citrate. The 2 solutions containing citrate yielded similar chromatograms, with no statistically significant difference between them in terms of peak area ( $p = 0.44$ ). The sample containing only gentamicin generated no peaks under the conditions of the assay. These results indicated that the presence of gentamicin in the sample did not affect the analysis of citrate.

## Stability Study

Four samples of each formulation were withdrawn at intervals over 112 days, stored frozen at  $-20^\circ C$ , and then analyzed for concentration of gentamicin and citrate using the validated HPLC methods described above. Analysis of all samples was complete by study day 126, so the longest storage time in the freezer was 126 days (for the samples obtained on day 0); the median storage time for the frozen samples was 98 days. All of the solutions retained at least 99% of the labelled concentration over time (Table 1). None of the chromatograms for either gentamicin or citrate showed the presence of unexpected peaks or peaks associated with the breakdown products seen in the forced degradation studies.

For each formulation, the concentration of drug present in the 4 samples obtained at each time point were compared by ANOVA. For formulation A (gentamicin 2.5 mg/mL), there was no significant change in gentamicin concentration over the 112-day study period ( $p = 0.60$ ). Similarly, for formulation B (sodium citrate 40 mg/mL), there was no significant change in citrate concentration over the same period ( $p = 0.57$ ). For formulation C (gentamicin and citrate combined), there was no change in the concentration of either gentamicin ( $p = 0.34$ ) or citrate ( $p = 0.055$ ) over the study period.

None of the formulations showed a significant change in either gentamicin or citrate concentration over the 112-day period (Table 1). This result was confirmed with regression analysis of concentration as a function of time: in all cases, the slope of the regression line was not significantly different from zero ( $p > 0.05$ ). From the linear regression analysis of the concentration–time data of formulation C, we calculated that 99.97% of the labelled amount of gentamicin and 101.3% of the labelled amount of citrate remained at day 112. The lower limit of the 95% CI also indicated that more than 98.17% of the gentamicin and more than 99.57% of the citrate remained on day 112.

The pH of each formulation did not change significantly from baseline to the end of the observation period ( $p > 0.05$  for all comparisons). The initial and final pH values were  $4.86 \pm 0.06$  and  $4.97 \pm 0.10$ , respectively, for formulation A;  $6.56 \pm 0.05$  and  $6.60 \pm 0.02$ , respectively, for formulation B; and  $6.47 \pm 0.03$  and  $6.50 \pm 0.02$ , respectively, for formulation C. At the end of the study period, samples of each formulation remained clear and colourless by visual inspection. The storage temperature was  $23.9 \pm 0.9^\circ C$  ( $n = 10$ ) over the course of the study.

## DISCUSSION

The HPLC methods used for analysis of gentamicin and citrate proved acceptable, and for both methods the validation studies indicated suitable linearity over an acceptable range, as

**Table 1. Concentrations of Gentamicin and Citrate for the 3 Study Formulations as a Function of Time, Expressed as Percentage of Label Content\***

Study Day	Formulation A (Gentamicin)	Formulation B (Citrate)	Formulation C	
			Gentamicin	Citrate
0	101.3 ± 2.27	103.3 ± 0.64	102.0 ± 0.75	102.8 ± 2.15
7	102.8 ± 0.87	104.3 ± 0.29	99.9 ± 1.64	101.5 ± 1.55
14	101.4 ± 3.19	104.6 ± 2.61	100.6 ± 0.13	102.2 ± 1.03
28	101.1 ± 1.94	103.9 ± 1.54	99.6 ± 0.60	101.8 ± 0.56
42	100.9 ± 1.30	104.2 ± 0.94	100.9 ± 1.73	102.6 ± 1.92
56	101.8 ± 2.30	104.4 ± 0.84	100.4 ± 2.08	100.8 ± 1.54
112	99.9 ± 1.13	103.4 ± 0.59	100.1 ± 1.86	101.6 ± 0.90

\*Values are means ± standard deviation (n = 4).

well as suitable accuracy and precision. The specificity of the methods was established by means of forced degradation, and the peaks for the degradation products all had retention times well separated from those of the analytes. There were no changes in peak symmetry or retention times for parent compounds over the validation period. The presence of citrate was shown to not interfere with the analysis of gentamicin, and the presence of gentamicin was shown to not interfere with the analysis of citrate.

For none of the formulations (gentamicin alone, citrate alone, gentamicin and citrate combined) were there any significant changes in concentration of either gentamicin or citrate over the 112-day study period.

## CONCLUSIONS

The results of this study indicate that an admixture of gentamicin and citrate at final concentrations of 2.5 and 40 mg/mL (4%), respectively, can be mixed and stored in polyethylene syringes and is stable at room temperature for at least 112 days with minimal change to the initial concentrations. In assigning a beyond-use date, however, sterility aspects of the product must also be considered, as outlined by the USP.<sup>16</sup> This information will allow extemporaneous compounding of this catheter lock solution in advance of use and will provide a stable product for use in clinical applications.

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**Dennis Cote**, BSc(Pharm), MSc, PhD, is an Instructor with the Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba.

**Charmaine E Lok**, MD, FRCPC, is Associate Professor with the Faculty of Medicine, University of Toronto, and Staff Nephrologist with the University Health Network—Toronto General Hospital, Toronto, Ontario.

**Marisa Battistella**, PharmD, ACPR, is a Clinical Pharmacist – Hemodialysis with the University Health Network—Toronto General Hospital, Toronto, Ontario.

**Lavern Vercaigne**, PharmD, is Associate Professor with the Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba.

**Address correspondence to:**

Dennis Cote  
Faculty of Pharmacy  
University of Manitoba  
750 McDermot Avenue  
Winnipeg MB R3E 0T5

**e-mail:** Dennis\_Cote@umanitoba.ca