ARTICLE

Stability of Trisodium Citrate 4.0% and 46.7% in Polyvinyl Chloride Syringes

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ABSTRACT

Objective: To evaluate the stability of solutions of trisodium citrate in saline, prepared under standard sterile pharmaceutical conditions and stored in 5-mL polyvinyl chloride (PVC) syringes.

Methods: Two concentrations of trisodium citrate, 4.0% and 46.7%, were tested. Samples were transferred to individual syringes, stored at room temperature (21°C), and kept away from sunlight. The samples were analyzed with a stability-indicating high-performance liquid chromatography method on days 1, 3, 7, 14, and 28.

Results: There was no significant decrease (less than 10%) in the trisodium citrate concentration in either solution, even after 28 days of storage.

Conclusion: Solutions of trisodium citrate both 4.0% (extemporaneously prepared) and 46.7% (commercially available), are stable for at least 28 days when stored at room temperature in PVC syringes.

Key words: stability, triCitrasol, trisodium citrate, anticoagulant

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RÉSUMÉ

Objectif : Évaluer la stabilité de citrate de sodium, dans des solutions salines entreposées dans des seringues de chlorure de polyvinyle de 5 mL et préparées selon les techniques pharmaceutiques stériles standard.

Méthodes : Deux préparations du médicament à des concentrations de 4,0 % et 46,7 % ont été étudiées. Les échantillons, mis dans des seringues individuelles et entreposés à la température ambiante (21 °C), à l'abri de la lumière du soleil, ont été analysés au jour 1, 3, 7, 14 et 28, au moyen d'une épreuve de stabilité par chromatographie liquide à haute pression.

Résultats : L'analyse n'a révélé aucune diminution significative (moins de 10 %) de la concentration en citrate de sodium des solutions à 4,0 % et à 46,7 %, même après 28 jours d'entreposage.

Conclusion : Les préparations extemporanées de citrate de sodium à des concentrations de 4,0 % et de 46,7 % dans des seringues de chlorure de polyvinyle sont stables pendant au moins 28 jours.

Mots clés : stabilité, triCitrasol, citrate de sodium, anticoagulant

INTRODUCTION

Heparin is a systemic anticoagulant commonly used for extracorporeal medical techniques such as hemodialysis. Bleeding is a frequent, sometimes life-threatening, complication of heparin therapy. An aqueous solution of trisodium citrate is an effective alternative anticoagulant.[1,2] In the extracorporeal system, trisodium citrate chelates the free ionized calcium in the blood to form a calcium citrate chelate, thus blocking the calcium-dependent steps of the coagulation cascade. When trisodium citrate reaches the bloodstream, a similar chelation process occurs between any free citrate and the large pool of free calcium in the blood. The liver and muscle then rapidly metabolize the chelate, allowing free sodium and calcium to enter the body's pool of ions. Trisodium citrate is therefore considered a regional anticoagulant that can be extremely useful when there is a high risk of bleeding.[1,2]

No previous studies of the stability of trisodium citrate in polyvinyl chloride (PVC) syringes have been published. Cytosol Laboratories has evaluated the stability of commercially available trisodium citrate 46.7% (triCitrasol) in glass bottles stored at ambient temperature.[3] That study demonstrated that triCitrasol remains stable for 3 years after the date of manufacture.

The purpose of the study reported here was to determine the short-term and long-term stability of 2 concentrations of trisodium citrate prepared and stored in PVC syringes; advance preparation and storage in syringes would facilitate delivery and dispensing of the drug.

METHODS

Commercially available trisodium citrate (triCitrasol; concentration 46.7%) and a 4.0% solution prepared by dilution with 0.9% sodium chloride were studied. The study was conducted from January to May 2000, ending roughly 18 months before the expiry date of the product.

High-Performance Liquid Chromatographic Analysis

The high-performance liquid chromatographic (HPLC) system consisted of an isocratic solvent pump (model 126 System Gold programmable solvent module, Beckman Instruments [Canada] Inc., Mississauga, Ontario) set at a flow rate of 1.2 mL/min, a diode array detector (model 168 System Gold diode array detection module, Beckman Instruments [Canada] Inc.) set at 215 nm, a manual injector (model Altex 210A valve, Beckman Instruments [Canada] Inc.) with a 100-µL fixed loop, and a C18 column with 5-µm particle size (model Ultrasphere ODS [octadecylsilane], 4.6 mm x 25 cm, Beckman Instruments [Canada] Inc.). The chromatographic method was based on official method 986.13 of the Association of Official Analytical Chemists.[4] The mobile phase was prepared by dissolving 13.6 g of monobasic potassium phosphate (American Chemical Society [ACS] grade, catalogue no. P-284, Fisher Scientific Company, Fairlawn, New Jersey) in about 950 mL of distilled, deionized water, adjusting the pH of the solution to 2.5 with concentrated (85% to 87%) phosphoric acid (ACS grade, catalogue no. 0260-03, J.T. Baker Inc., Phillipsburg, New Jersey), and diluting to 1 L.

During assay validation and on each study day, a stock solution of trisodium citrate (AnalaR grade, catalogue no. B10242, BDH Inc., Toronto, Ontario) was prepared by dissolving 200 mg of the salt in water and diluting to 100 mL with distilled, deionized water. The concentration of this stock solution was 2.00 mg/mL. All trisodium citrate standards were prepared by appropriate dilution of the stock solution. Five standards, ranging in concentration from 0.005 to 0.200 mg/mL, and a blank were used to prepare each calibration curve. All standards were chromatographed in triplicate. The 0.100 mg/mL standards were also used to assess recovery of trisodium citrate from the column and to determine intra-day and inter-day reproducibility.

Assay Validation

To verify the suitability of the HPLC method as a stability-indicating assay, a 4.0% solution of trisodium citrate was prepared by dissolving 20 g of the salt in water and diluting to 500 mL with distilled, deionized water. The pH of two 100-mL aliquots was adjusted to approximately 1 (with concentrated hydrochloric acid) and 12 (with 6N sodium hydroxide), respectively. A third 100-mL aliquot was treated with 50 mL of 30% hydrogen peroxide. These 3 degradation samples were heated under reflux at a temperature of about 100°C for 24 h. After heating, the pH of each solution was adjusted to about 7 with either concentrated hydrochloric acid or 6N sodium hydroxide, to match the pH of the trisodium citrate samples used for the stability study and to conform with chromatographic conditions. Each sample was diluted to 0.100 mg/mL trisodium citrate and analyzed by HPLC. The resulting chromatograms were inspected for the appearance of any peaks not corresponding to trisodium citrate. Fresh and degraded samples were compared for any change in concentration, retention time, and peak shape. Ultraviolet spectra were obtained with the photodiode array, with wavelengths ranging from 190 to 390 nm at 1-nm intervals; spectral overlay allowed comparisons of fresh and degraded samples.

Intra-day and inter-day reproducibility of samples was determined by coefficient of variation (CV), which was defined as the standard deviation divided by the mean multiplied by 100. A recovery study was also

performed to determine the accuracy of the method. Solutions of 0.100 mg/mL prepared from precisely weighed samples of trisodium citrate were analyzed by HPLC, and concentrations determined from the standard curve were compared with theoretical values. Accuracy was taken as the percent deviation of concentrations, determined by back-calculation from the standard curve, from known concentrations. Each sample injected into the column was carefully monitored and compared with another injected sample for the same sample or standard. If any discrepancy was found, additional samples were injected and analyzed.

The linearity of detector response was assessed by the correlation coefficient of the slope of the 6-point calibration curve (triplicate samples of concentrations ranging from 0 to 0.200 mg/mL). Because the concentrations of the samples were of the order of 0.100 mg/mL, the concentrations of the calibration curve ranged from 0 to 200% of expected values. The sensitivity of the HPLC assay was evaluated to determine (from the signal-to-noise ratio) the minimum amount of trisodium citrate that could be measured by this method. The signal-to-noise ratio of 30 different aliquots of a blank (0.9% sodium chloride in water) was used to calculate sensitivity. Blanks were also used to determine the intercept of the standard curve and assisted in the detection of any interfering peaks.

Stability Study

The contents of 30-mL glass vials of triCitrasol 46.7% (catalogue no. PN-6030, manufactured for Citra Anticoagulants Inc. by Cytosol Laboratories, Inc., and distributed by Medcomp, Harleysville, Pennsylvania; lot M99B, expiry date November 30, 2001) were either diluted to 4.0% with 0.9% sodium chloride, to reflect usual preparation of an isotonic solution, or left undiluted. Samples were transferred to 5-mL PVC syringes (catalogue no. 309634, Becton-Dickinson, Rutherford, New Jersey) with caps (catalogue no. 7710RC, Acacia Inc., Loma Linda, California). Fifty samples of each concentration were stored at room temperature and kept from direct exposure to light. On days 1, 3, 7, 14, and 28, samples from 10 randomly selected syringes of each concentration were diluted with distilled, deionized water to yield concentrations of 0.100 mg/mL, and the resulting solutions were analyzed by HPLC. For the 4.0% samples, a 250-µL aliquot was diluted to 100 mL with distilled, deionized water. For the 46.7% concentration, it was necessary to perform 2 dilutions. The first step consisted of diluting the 46.7% solution to 4.0% with distilled, deionized water by taking an 857-µL aliquot of the concentrated sample and diluting to a volume of 10 mL. The second step involved taking a 250-µL aliquot of the solution prepared from the 46.7% sample and diluting it to 100 mL with distilled, deionized water.

On the first day, an additional 10 samples of each concentration were placed in storage at –70°C to represent the solutions on day 1 of the study. On each day of analysis, 2 of these frozen samples were thawed, diluted to 0.100 mg/mL, and analyzed by HPLC. In addition, a freshly prepared trisodium citrate solution (0.100 mg/mL) was used as a control. On each day of analysis, these trisodium citrate standards (day 1 and control) were analyzed by HPLC. Each sample was analyzed in duplicate.

Means (± standard deviation) were calculated from the results obtained by HPLC. A limit of no greater than 10% decrease from the original concentration was chosen to indicate stability.

RESULTS

Assay Validation

The trisodium citrate solution with pH adjusted to about 1 and the solution treated with hydrogen peroxide had no visible change in appearance after heating for 24 h. However, the solution with pH adjusted to 12 had a milky white, gelatinous precipitate after 24 h. The alkaline solution was filtered to protect the column from any residue. HPLC analysis of the acid-treated and peroxide-treated solutions showed at least 1 and possibly 3 additional peaks (Figures 1c and 1d). Conversely, the trisodium citrate peak was reduced in height, which indicates degradation of the sample. HPLC of the alkali-treated solution showed no additional peaks, but the height of the trisodium citrate peak was lower than expected. This decrease might be attributable to degradation of the sample and also to the filtering process (Figure 1b). None of additional peaks interfered with the detection of trisodium citrate. Spectral overlays of trisodium citrate and the presumed degradation peaks showed some similarity, which suggests that the additional peaks represented degradation products. Similarly, comparison of spectra for trisodium citrate and triCitrasol demonstrated agreement.

The measured parameter for the calibration curve was peak height. The detector response curve (calibration curve) was linear over the range studied here, with a correlation factor (r^2) of 0.9998. Analysis of blanks showed that the calibration curve intercepted the origin. The sensitivity of the assay was determined to be 480 ng (0.0048 mg/mL) by signal-to-noise estimates over 30 different blanks. The intra-day and inter-day variances were 0.99% and 2.64%, respectively. The average recovery value was 101.5% ± 1.2% of the expected value (0.100 mg/mL).

Stability Study

During the course of the study, the solutions of

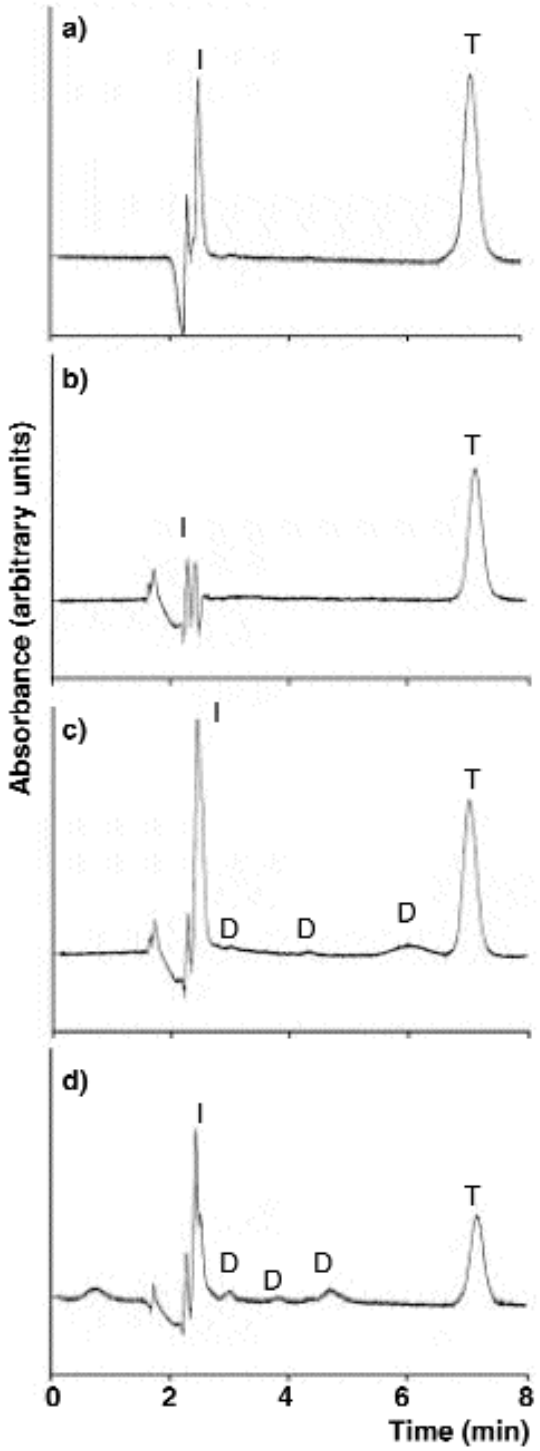

Figure 1: Chromatograms of degradation samples of trisodium citrate in water. T = trisodium citrate, I = injection, D = presumed degradation product. (a) Fresh solution of trisodium citrate. (b) Trisodium citrate after adjustment to pH 12 with 6N sodium hydroxide and reflux for 24 h at 100°C. (c) Trisodium citrate after adjustment to pH 1 with concentrated hydrochloric acid and reflux for 24 h at 100°C. (d) Trisodium citrate after mixing with 50 mL of hydrogen peroxide and reflux at 100°C. For all chromatograms, conditions were identical (absorbance 215 nm, 100-µL injection volumes), as are the time scales shown.

Table 1. Stability of Trisodium Citrate Prepared in 0.9% Sodium Chloride and Stored at 21°C

Study Day	% of Initial Concentration Remaining*	
	4.0% Solution†	46.7% Solution‡
1	Baseline	Baseline
3	90.2 ± 2.7	101.7 ± 3.3
7	95.1 ± 2.9	101.0 ± 2.9
14	92.0 ± 3.0	93.7 ± 2.4
28	90.0 ± 2.1	96.8 ± 1.1

*Mean ± standard deviation of 10 samples. Each sample was analyzed in duplicate.
†Actual initial concentration 4.0% ± 0.1%.
‡Actual initial concentration 46.7% ± 1.7%.

trisodium citrate stored in PVC syringes remained clear, colourless, and free of any precipitate. There was no definitive trend for a decrease in concentration over the 28-day study period. Relative to day 1 concentrations, the average percent remaining was 90.0% or greater on all study days (Table 1).

DISCUSSION

At the time this study was done, trisodium citrate was commercially available only as a 46.7% solution in 30-mL vials[5] [see "Note added in proof", at the end of this article — Editor]. Smaller volumes (2.1 mL) and a lower concentration (4.0%) are used in the clinical setting. The objective of this study was to evaluate the stability of trisodium citrate in PVC syringes, since advance preparation of the drug would improve its practicability.[1,6]

Factors that may influence the degradation of any drug or chemical substance are light, heat, and pH, as well as presence of acids, alkalis, oxidizing agents, and atmospheric oxygen. In the assay validation portion of this study, various combinations of heat and acid, alkali, and oxidizing agent were applied to a series of aliquots of a 4.0% aqueous solution of trisodium citrate (the degradation samples). Trisodium citrate stability was affected by all combinations of heat with acid, alkali, or oxidizing agent, although the extent of degradation varied with the experimental conditions (acidic, alkaline, or oxidizing). Oxidizing conditions (Figure 1d) caused the most degradation (greatest decrease in peak height), followed by alkaline conditions (Figure 1b) and acidic conditions (Figure 1c). The appearance of additional peaks in the solutions subjected to acidic and oxidizing conditions was presumed to indicate the presence of degradation products of trisodium citrate. If the degradation samples were not refluxed, the extent of degradation was much lower and the resulting chromatograms closely resembled those of fresh solutions, with almost no decrease (or no discernible decrease) in peak height.

On day 1 of the stability study 10 samples of each concentration were frozen at –70°C. In a previous study (unpublished), samples of 4.0% and 46.7% trisodium citrate stored frozen at –70°C lost less than 1% of their initial concentration over 28 days, and their detector response once thawed was unchanged. It was therefore possible to use a frozen sample on each study day as a quality control sample that could be compared to the 0.100 mg/mL trisodium citrate standard. This ensured consistency on each day of analysis.

During the stability study, the syringes containing the samples were protected from light. During packaging, care was taken to introduce as little air as possible into the syringes. Therefore, although levels of light and atmospheric oxygen were not evaluated, these factors probably had little bearing on stability over the 28-day study period. No attempts were made to control or adjust the pH of the trisodium citrate samples. For these samples, any degradation could not have been due to heat (because samples were kept at room temperature) or to acids, alkalis, or oxidizing agents (because such reagents were not added during the stability study). Since at least 90% of the initial concentration of trisodium citrate remained on day 28, it can be concluded that these storage conditions did not lead to degradation.

The concentration of the sample solutions had a small effect on their stability. The concentrated (46.7%) samples were somewhat more stable, retaining 96.8% of the initial concentration by day 28, whereas the dilute (4.0%) samples retained only 90.0% of the initial concentration.

This study demonstrated that 4.0% solutions of trisodium citrate packaged in capped PVC syringes under less than optimum conditions (where optimum conditions would consist of packaging under nitrogen, with pH adjusted to about 6.7, as recommended by Citra Anticoagulants[5]) were stable for at least 28 days when stored at room temperature and protected from light. More concentrated (46.7%) solutions of trisodium citrate packaged in the same manner were also stable for at least 28 days.

NOTE ADDED IN PROOF

The brand of prepared trisodium citrate solution used in this study, triCitrasol, was recalled in April 2000 for all uses with blood access catheters.[7] The product was recalled because it had been marketed without proper marketing clearance from the US Food and Drug Administration and because of a potential public health hazard when used at concentrations greater than 4%. At that time, the distributor, MedComp, advised that there would be no further marketing or distribution of triCitrasol for any use. The experimental work for this study was completed at about the time of the recall. The report is being published for the benefit of hospital pharmacies and laboratories that may be preparing their own solutions of trisodium citrate and may wish to have information concerning the stability of such preparations.

References

1. Grant ME, Lowell HB, Wiegmann T. Current use of anticoagulation in hemodialysis. *Semin Dialysis* 1991;4:168-73.
2. Ouseph R, Ward RA. Anticoagulation for intermittent dialysis. *Semin Dialysis* 2000;13:181-7.
3. Cytosol Laboratories Inc. *Final report for the stability of triCitrasol® in ambient storage conditions for three year stability dating.* Braintree (MA): The Laboratories; 2001.
4. Association of Official Analytical Chemists International. *Official methods of analysis*, 16th edition [CD-ROM version]. Gaithersburg (MD): The Association; 1998.
5. Medcomp. triCitrasol product monograph. Harleysville (PA); 1999: November.
6. Buturovich J, Ponikvar R, Kandus A, Boh M, Linkmann J, Ivanovich P. Filling hemodialysis catheters in the interdialytic period: heparin versus citrate versus polygeline: a prospective randomized study. *Artif Organs* 1998;22:945-7.
7. Recall of Citra Anticoagulants, Inc. triCitrasol anticoagulant sodium citrate concentrate (46.7% trisodium citrate) Citra part number pn-6030 (30ml.) also distributed by Medcomp as triCitrasol anticoagulant sodium citrate concentrate (46.7% trisodium citrate) catalog number PN6030. Harleysville (PA): Medcomp; 2000 Apr 14. Available from: http://www.medcomp-net.com/ (accessed 2001 Oct 16).

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