Purpose: To compare 3 methods for creating ethylenediaminetetraacetic acid (EDTA) solution using readily available Vacutainer tubes for the treatment of band keratopathy.

Methods: All 3 protocols used commercially available Vacutainer blood collection tubes coated with K$_2$EDTA. An osmometer was used to measure and compare the concentration of EDTA created using 3 different protocols. The time required for preparation of the solution was measured and compared to evaluate its efficiency for everyday clinical use. In addition, volume of EDTA solution obtained was measured for method 1. The most promising protocol for clinical use was then used for treatment of a series of patients.

Results: Average osmolarity was 532, 285, and 422 for methods 1, 2, and 3, respectively (ANOVA $P < 0.01$, all Tukey honestly significant difference $P < 0.01$). For the respective mixtures, average concentration was 65, 35, and 52 mg/mL, and average time to create solution was 189, 38, and 83 seconds (ANOVA $P < 0.01$, all Tukey honestly significant difference $P < 0.01$). The most promising, method 3, was found to be safe and effective in removing calcium from the corneal stroma in a series of 5 patients with 6 eyes treated. It also yielded 25% more solution for clinical use than method 1.

Conclusions: Method 3 using a single 10-mL Vacutainer tube with 18 mg of K$_2$EDTA had the best balance of effective concentration of EDTA, time to preparation, and simplicity of methodology, when compared with previously published methods 1 and 2. It also yielded a greater final volume of solution.

Key Words: band keratopathy, EDTA, ethylenediaminetetraacetic acid, chelation, treatment

Calcific band keratopathy (CBK) is a degenerative condition of the cornea that primarily affects Bowman layer. Fine grayish-white calcium opacities form a horizontal band toward the central cornea and, as the condition progresses, can cause loss of vision and increased glare and result in corneal erosion-like symptoms and ulceration. A variety of conditions increase susceptibility to CBK: corneal exposure, calcium-phosphate imbalance, alteration in tear osmolality, alkalosis, and chronic ocular inflammation.

Ethylenediaminetetraacetic acid (EDTA) is the most widely used method of treating CBK. EDTA is a chelating agent that is commonly used as a food additive and in cosmetic products and can also be found in standard blood collection tubes where it is used as an anticoagulant. EDTA for ophthalmic use was originally derived from Na$_2$EDTA but that formulation has lost approval from the FDA and can now only be obtained at specially equipped compounding pharmacies. It is recommended to treat CBK with a 3% to 4% solution of disodium EDTA (concentration = 30–40 mg/mL).

Lee et al proposed a novel and useful method of producing EDTA by dissolving the K$_2$EDTA lining found in blood collection tubes. Using this method, the authors discovered that, although effective, preparation was complex, relatively time consuming, and yielded only a small amount of usable solution due to transfer loss through 5 different tubes. This often necessitated repeating the process to obtain enough EDTA solution to treat a patient. We sought to create a protocol that created an EDTA solution of at least the currently accepted concentration that was easier and faster to create than the previously published protocol. We tested the method of Lee et al and 2 other methods of producing a solution of EDTA for treatment of CBK and found one that is significantly faster and simpler yet yields an effective concentration of EDTA with a larger volume to allow for multiple applications.

MATERIALS AND METHODS

We compared 3 methods of EDTA preparation. In method 1, outlined by Lee et al, 0.3 mL of sterile water was injected into a purple-topped 3-mL K$_2$EDTA Vacutainer blood collection tube (Becton, Dickinson and Company, Franklin Lakes, New Jersey).
Lakes, NJ) containing 5.4 mg of K$_2$EDTA. The diluent was swirled to dissolve the K$_2$EDTA coating the inside of the tube. The solution was then transferred from tube to tube, and the process was repeated for 5 tubes. The end solution volume was measured after repeating the process 5 different times.

In method 2, 0.3 mL of sterile water was injected into a single 10-mL K$_2$EDTA Vacutainer blood collection tube containing 18 mg of K2-EDTA was used (Becton, Dickinson and Company) and swirled to dissolve the K$_2$EDTA coating the inside of the tube. In method 3, the tube cap was removed, and 0.3 mL of sterile water was injected into the 10-mL K$_2$EDTA tube containing 18 mg of K$_2$EDTA, then a sterile, dry cotton-tipped applicator was used to absorb the fluid in the tube before then sweeping along the entire interior surface of the tube to dissolve the K$_2$EDTA coating the inside (see Video, Supplemental Digital Content 1, http://links.lww.com/ICO/B153, which demonstrates method 3). The end point was absence of a gritty sensation from the K$_2$EDTA salt as the cotton-tipped applicator rubbed and dissolved the EDTA.

Ten trials were repeated for each method. Osmolarity was measured with the Osmo1 Single-Sample Micro-Osmometer from Advanced Instruments (Norwood, MA). Residual fluid at the bottom of the tubes was sampled in all 3 methods. The preparation time for each of the 10 trials in the 3 different methods was also measured. The results were averaged within their respective method. Differences between the 3 methods were analyzed using ANOVA and Tukey honestly significant difference (HSD) tests from astatsa.com for post hoc analysis.

Method 3 had the best characteristics for clinical use, and it was used in a series of patients who gave informed consent for treatment of their band keratopathy. This study conformed to the ethical principles found in the Declaration of Helsinki.

### RESULTS

Using method 1, the average osmolarity was 531.7 mOsm/L (SD = 34.3; calculated concentration: 65.30 mg/mL). The preparation time took, on average, 188.7 seconds (SD = 18.9), and the average pH was 5.

Using method 2, the average preparation time was 38.4 seconds (SD = 6.6). The average osmolarity was 285.3 mOsm/L (SD = 86.7; calculated concentration 35.04 mg/mL), and the average pH was 4.25.

Using method 3, average preparation time was 83.1 seconds (SD = 8.8), and average osmolarity was 421.9 mOsm/L (SD = 67.4; calculated concentration 51.81 mg/mL); the average pH was 4.5. The results are summarized in the Table 1.

There were significant differences when comparing osmolarity [ANOVA F(2,27) = 34.5, P < 0.01; Tukey HSD, all P < 0.01] and preparation time (ANOVA F(2,27) = 374.6, P < 0.01; Tukey HSD, all P < 0.01) between the 3 methods.

We found clinically significant differences in preparation time between methods 1 and 3, but no clinically significant differences in concentration between methods 1 and 3.

Method 1 had an average yield of 0.24 mL of solution. Method 3 had, on average, 25% more volume for clinical use than method 1.

All patients treated with method 3 experienced no complications, and no delayed healing. Figure 1 shows photographs of 1 patient before and after treatment with EDTA produced with method 3. Uncorrected visual acuity before and after chelation therapy for our series of patients is summarized in Table 2.

### DISCUSSION

K$_2$EDTA has been shown to be an effective alternative to Na$_2$EDTA, which is both expensive and inconvenient to obtain. It is also cost-effective and widely available. At the time of writing, we found that the average cost of an ophthalmic preparation of Na$_2$EDTA was $117 (range $70–$195), whereas 10-mL blood collection tubes cost $35.99 to $46.99 per 100 tubes.

Method 1 for producing K$_2$EDTA as outlined by Lee et al took a substantial amount of time and had the greatest complexity. It had the highest concentration solution but less volume for use. Method 2 was the fastest to prepare but yielded the lowest concentration of K$_2$EDTA. Method 3 struck a good balance with a greatly reduced the preparation time, greater simplicity of preparation, while achieving a higher concentration of EDTA than that commonly used to treat CBK (3%–4% disodium EDTA with concentration of 30–40 mg/mL). Method 3 also yielded greater volumes of solution than that by method 1, because method 1 requires transfer of fluid between 5 different vials, and residual fluid remains in the walls of the vials after transfer that cannot be

### TABLE 1. Average Osmolarity With SD, Calculated Concentration, and Preparation Time With SD for 3 Methods of Creating “Off-the-Shelf” EDTA Solution, With Standard Treatment as Comparison

| Method      | Mean osmolarity (±SD), mOsm/L | Concentration (mg/mL)* | Mean preparation time (±SD) (s) |
|-------------|-------------------------------|------------------------|---------------------------------|
| Method 1    | 531.7 (±34.3)                 | 65.3                   | 188.7 (±18.9)                  |
| Method 2    | 285.3 (±86.7)                 | 35.04                  | 38.4 (±6.6)                    |
| Method 3    | 421.9 (±67.4)                 | 51.81                  | 83.1 (±8.8)                    |
| 3% EDTA Solution | 244.29                       | 30                     |

*aOsmolarity was converted to concentration of EDTA in mg/mL using the equation: mOsm/L × 1/368.42 mg/mmol × 1L/1000 mL.*

### FIGURE 1. Photographs before (left) and after (right) treatment with EDTA derived from method 3. (The full color version of this figure is available at www.corneajrnl.com.)

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retrieved. Method 3, most importantly, had good clinical efficacy. We found its clinical effectiveness indistinguishable from our previous clinical experience with method 1, and our impression is that both methods 1 and 3 are considerably faster in removing CBK than using the standard compounded 3% EDTA.

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