

# Broad-Beam Ultrasound for Acceleration of Struvite Calculi Dissolution Using Citric Acid-Based Chemolytic Agents

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## ABSTRACT

**A computer-controlled broad-beam ultrasound system is presented that accelerates struvite stone chemolysis *in vitro*. Hemiacidrin chemolysis was greatly potentiated and was linearly dependent on stone surface area and total ultrasound time. Dissolution also was dependent on citric acid concentration and solution pH, with only minimal dissolution above pH 4.2. Citrate concentrations of 12% provided maximal dissolution that was further potentiated by 0.1 M EDTA. With or without ultrasound application, the citrate-based compound demonstrated eight-fold greater activity than hemiacidrin. Magnesium competitively inhibited dissolution.**

## INTRODUCTION

**R**ECENT ADVANCES in endourologic techniques have created new therapeutic challenges, particularly relating to the percutaneous treatment of struvite calculi, as multiple small fragments are often left in the kidney after attempts at stone removal. Removal of all fragments is important, because any remaining struvite may harbor causative bacteria. Because of the difficulty in achieving a stone-free state with either extracorporeal shock wave lithotripsy (ESWL) or with percutaneous techniques, a renewed interest in chemolytic agents has developed. Herein, we utilize a new technology to investigate the kinetics of stone dissolution, and we describe the use of a new chemolytic agent developed using this technology. The unique application of high-intensity, broad-beam ultrasound accelerates stone chemolysis, probably through a microcavitation process, thereby allowing rapid study of multiple stone-dissolution parameters in a fraction of the time required with more conventional water-bath stirring devices.

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## MATERIALS AND METHODS

A laboratory ultrasound device was used to study the effect of high-intensity ultrasound on human struvite kidney stones. The ultrasonic transducer was driven by an amplified calibrated radiofrequency source. Multiple frequencies and amplitudes were investigated to find optimal parameters, and all experiments were conducted at the same fixed energy output at the focal point, as calibrated by a steel ball deflection device.

Human kidney stones were obtained from the Methodist Hospital of Indiana Institute for Kidney Stone Disease in Indianapolis and Beck Analytical Services in Bloomington, Indiana. Stone composition was determined by Beck Services, and stones ranged from 60 to 100% in total struvite composition. Stone size ranged from 200 to 1500 mg; the average size was 570 mg. Small fragments obtained during ultrasonic lithotripsy were also studied.

Stones were placed in a latex finger cot containing 7 ml of chemolytic agent at various concentrations and pH (Table 1). The stone was then fixed at the focal point of the ultrasound beam. The transducer and stone were placed in a 37°C degassed water bath. A corresponding matched control stone also was placed in the water bath outside the ultrasound field. Absolute weight loss of both control and experimental stones was assessed to 1 mg at various time intervals before total dissolution.

To determine if citrate buffer plus EDTA would have greater activity than hemiacidrin in the absence of ultrasound potentiation, stones ranging in weight from 1.7 to 0.059 g were placed in a latex finger cot containing CBE and softly agitated in a 37° water bath for 2 to 24 hours.

All chemicals except hemiacidrin were reagent grade and obtained from Sigma Chemical; hemiacidrin was obtained from Guardian Chemical Company under the trade name of Renacidin. Solutions were prepared fresh daily using degassed water. Ethylenediaminetetraacetic acid (EDTA) was used in anhydrous form.

The rate of stone chemolysis was represented graphically either by applying absolute mass loss of stone (g) against elapsed ultrasound time or by calculating a percentage of initial stone weight. Observed variability in the data suggested a surface-area effect. Further investigation using both single stones and multiple small fragments (to maximize surface area) demonstrated a linear relation between absolute weight loss and stone size. Therefore, to account for variability secondary to surface area, data were represented as a percentage of initial stone size, which uniformly decreased the standard error of the mean. Chemolysis was measured at a fixed elapsed ultrasound time of 24 minutes in all experiments. Two sample t-tests were performed for data comparison, and significance was established at  $p < 0.01$ . Data points were represented as the mean values  $\pm 1$  S.E.M.

## RESULTS

Initial experiments were conducted to determine if chemolysis could be accelerated by application of ultrasound. In Figure 1, the absolute weight loss of struvite stones treated with ultrasound in a solution of 5% hemiacidrin (pH 3.9) is plotted, as is the dissolution of the control stone (treated without ultrasound). During 24 minutes of ultrasound exposure, experimental stones ( $N = 9$ ) lost  $39 \pm 11$  mg (7% of its original weight), whereas control stones lost an average 0.5 mg. This 75-fold increase in the dissolution rate demonstrated the applicability of ultrasound to the study of stone chemolysis.

To investigate the mechanism of hemiacidrin action on struvite stones, dissolution was first measured as a function of hemiacidrin concentration. Concentrations from 2.5 to 15% were studied, because concentrations within this range are used clinically. A linear increase in stone dissolution with increasing hemiacidrin concentration was found (Fig. 2). The dissolution rate for 10% hemiacidrin was three times that for 5% hemiacidrin. This three-fold increase in activity could be explained by either a pH effect, an increase in concentration of the components of the solution, or both. An inverse relation exists between hemiacidrin solution concentration and pH. For example, 5% hemiacidrin contains 2.8% citric acid, final pH 3.9. A 10% hemiacidrin solution contains 5.6% citric acid, pH 3.6. The pH versus concentration effect was investigated by measuring dissolution at fixed pH while varying the hemiacidrin concentration (Fig. 3). These data demonstrate the dissolution of struvite calculi to be markedly sensitive to pH and, to a lesser

## ULTRASOUND IN STRUVITE CALCULI DISSOLUTION

TABLE 1. CHEMOLYTIC AGENTS STUDIED

	<i>pH</i>
5% hemiacidrin	3.9
10% hemiacidrin	3.6
12% citrate	3.9
12% citrate	3.6
12% citrate + 0.1 M EDTA	3.9
12% citrate + D-gluconic acid	3.9
12% Citrate + 0.1 M EDTA + Mg oxide	3.9

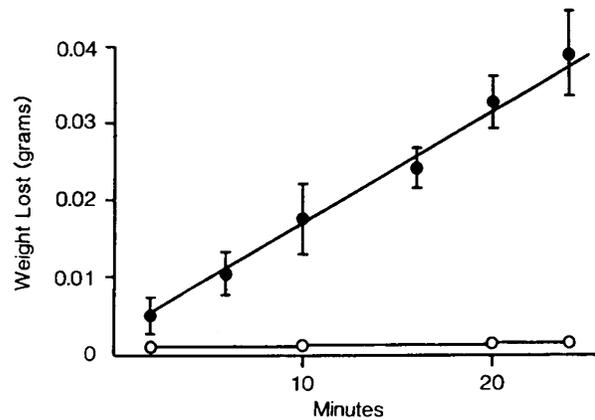


FIG. 1. Struvite calculi dissolution in 5% hemiacidrin (pH 3.9). ● ultrasound, ○ no ultrasound; n = 5.

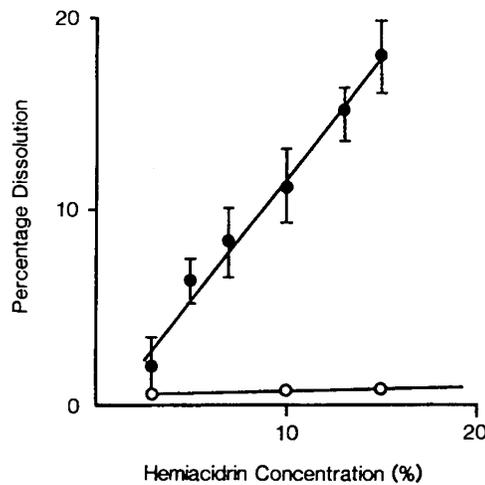


FIG. 2. Dissolution of struvite stones with various hemiacidrin concentrations; n = 3, mean  $\pm$  SEM, ● ultrasound, ○ no ultrasound.

extent, to hemiacidrin concentration. The relation between a standard concentration of hemiacidrin and pH was examined by varying the pH from 2.0 to 3.5 and 4.5 to 7.5 at 0.5 increments. At pH >4.0, the dissolution activity of hemiacidrin decreases, with no significant dissolution above control values at pH >4.5. There was exponential increase in dissolution rate from 3.6 to 2.0 with a peak dissolution rate of 70

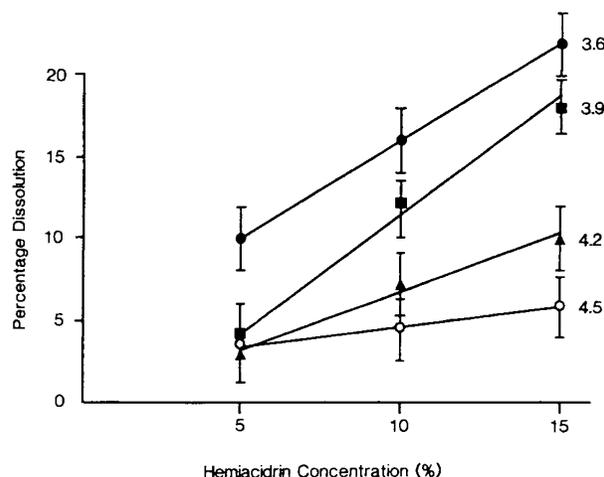


FIG. 3. Struvite stone dissolution at various concentrations of hemiacidrin at different pH.

$\pm 9\%$  (24-minute ultrasound) at pH 20.0. This dissolution rate was independent of hemiacidrin concentration. This high dissolution rate remains of only theoretical interest, as the caustic effect on the urothelium would preclude any clinical applicability at pH  $< 3.5$ .

The role of citrate within the hemiacidrin solution was next studied. As noted from Figure 3, citric acid appears to have intrinsic dissolution properties independent of the simple liberation of hydrogen ion and subsequent neutralizing of the ammonium component of the struvite stone. To investigate further the effect of citric acid on stone dissolution, pH was controlled at 3.6, 3.9, and 4.0; and at each pH, the citric acid concentration was varied from 5 to 20% (Fig. 4). The solutions were buffered using sodium carbonate and calcium carbonate initially, as described by Suby and Albright and by Mulvaney. However, these buffers were inadequate in that the liberation of  $\text{CO}_2$  during mixing made a degassed state impossible, thereby interfering with ultrasound delivery. Moreover, carbonate compounds are difficult to dissolve and to titrate accurately. Five-molar sodium hydroxide was used instead. Control studies demonstrated a small (2%) but statistically significant increase in the dissolution rate with this buffer, probably secondary to improved sound transmission and decreased competitive inhibition by calcium for citric acid.

Figure 4 demonstrates a linear increase in ultrasound-mediated dissolution with increasing citric acid concentration at all pHs studied. The peak dissolution activity occurs at approximately 12% citric acid. Above this concentration, there is slight increase in activity; however, the difficulty in buffering becomes significant. At pH 3.9, 12% citrate demonstrates a dissolution rate of 31% compared with a 4% dissolution rate for 5% hemiacidrin at pH 3.9. This eight-fold increase in dissolution rate at pH 3.9 is indicative of the intrinsic ability of citric acid to stimulate stone dissolution.

D-glutamic acid also has been described as an active dissolution agent for struvite stone. When 1.5% D-glutamic acid was added to 4% citrate buffer at pH 3.9, there was no significant increase in dissolution activity (Fig. 5). At 5% citrate concentration, D-glutamic acid did potentiate chemolysis by 2%.

Several authors have suggested that EDTA would potentiate calcium stone dissolution by chelating the calcium component. Because EDTA also is a magnesium-chelating agent, we examined the effect of the addition of EDTA to the 12% citrate buffer. Various EDTA concentrations were examined; however, only at 0.05 M was stone dissolution potentiated. At 0.1 M EDTA, the dissolution rate increased to 38% at pH 3.9 (24 minutes' elapsed ultrasound time).

Magnesium oxide also has been used as a component of chemolytic solutions. Therefore, magnesium ion was added in concentrations similar to that used in hemiacidrin to 12% citrate buffer with and without EDTA. In both cases, magnesium significantly decreased the dissolution rate, to 25 and 27%, respectively (Fig. 5).

When the citrate buffer plus EDTA was compared with 10% hemiacidrin without the application of ultrasound, the average weight loss in 24 hours was  $0.729 \pm 0.084$  g in the citrate buffer plus EDTA and

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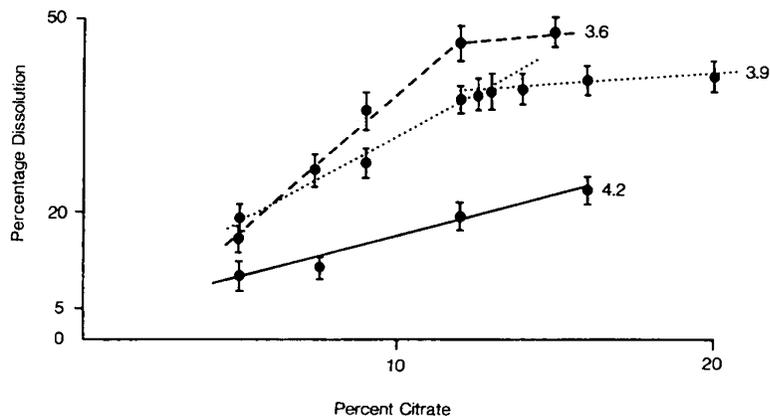


FIG. 4. Struvite stone dissolution rate at increasing citrate concentration at fixed pH.

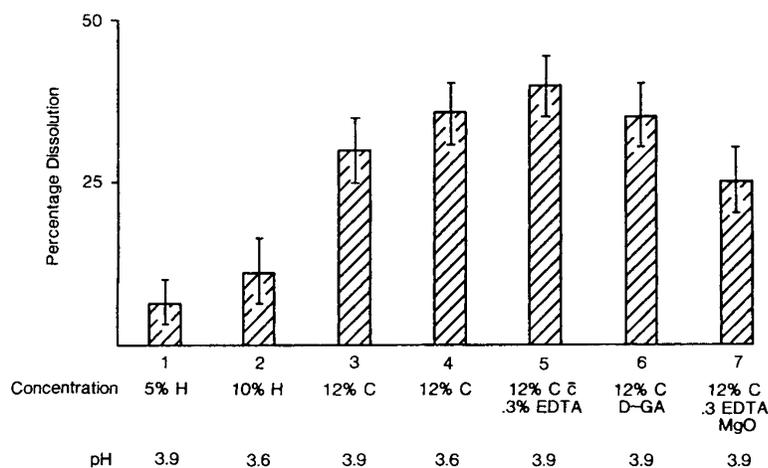


FIG. 5. Dissolution rates for struvite calculi using various chemolytic solutions. Ultrasound time = 24 minutes.

0.091 ± 0.018 g in hemiacidrin. There was linear increase in weight loss over 24 hours for both solutions. At each 2-hour time interval, there was approximately an eight-fold increase in activity of the citrate buffer plus EDTA compared with hemiacidrin. Because struvite density is approximately 0.25 g/cm<sup>3</sup>, this would correspond to dissolution of a 2-cm<sup>3</sup> stone over 24 hours.

## DISCUSSION

The data presented above demonstrate that the action of hemiacidrin is dependent on both the pH of the solution and the absolute citrate concentration. Further, the action of EDTA can enhance the chemolytic activity of citric acid buffer. The mechanism of action of EDTA most likely represents a chelation phenomenon, and the observation that magnesium oxide decreases the activity of the buffer is not surprising, as magnesium would be competing for ionic binding sites on the EDTA and citrate molecules. The addition of magnesium-based compounds for dissolution seems contradictory, since during dissolution, there is probable supersaturation of the solution with magnesium ion from the breakdown of the struvite crystal. In theory, adding magnesium ion to the dissolution agent would increase competitive inhibition and decrease the buffer activity, a concept supported by the data of this study. Moreover, the presence of magnesium has been responsible for significant toxicity due to the excessive absorption of magnesium during irrigation, as described by Thompson and Mora.

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A better buffer for the dissolution of struvite calculi may be 12% citric acid with 0.1 M EDTA, with pH adjusted to 3.9 with 5 M sodium hydroxide; the solution should be free of added magnesium. Although there would be significantly greater activity at pH 3.6, the urothelium is easily irritated by hydrogen ion concentrations in this range, and although 10% hemiacidrin has a pH of 3.6, it is likely that a solution at pH 3.9 would be tolerated better clinically.

The ultrasound technology is as important as the buffer. Ultrasound allows rapid investigation of dissolution by accelerating chemolysis. In so doing, significant dissolution can be achieved in minutes rather than hours or days, thereby allowing rapid screening for and comparison of subtle changes in solution composition. These data also demonstrate that a compound developed using ultrasound will eventually work even without ultrasound potentiation. This technology could easily be used to study other stone types such as cystine, uric acid, and calcium oxalate. Finally, the possibility for clinical use of broad-beam ultrasound to potentiate chemolysis merits further investigation, as it may represent a valuable adjunct to percutaneous lithotomy and ESWL, and it has the potential to be used as primary therapy in high-risk patients.

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