Acta Urol. Jap. Vol. 26
No. 3, March 1980

ISOTACHOPHORESIS FOR THE ANALYSIS OF URINARY TRACT STONE: A PRELIMIRARY REPORT

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ABSTRACT

Newly devised isotachophoretic apparatus was used for the analysis of urinary tract stones and was found to be useful for rapid and simple quantitative analysis.

INTRODUCTION

It is important to determine the composition of urinary tract stone for understanding the mechanism of stone formation and preventing recurrence. Infrared spectroscopy [3,11], X-ray defraction[9,10], optical crystallographic analysis[9] and other methods have been used for this purpose. These methods are essentially qualitative or semiquantitative. Chemical analysis of stones[5,8] is not easy because it required somewhat larger specimen and the sample must be separately processed to determine each component such as calcium, magnesium, oxalate, phosphate, urate, and so on. A sensitive, rapid, simultaneous, easy and inexpensive method is desired for the quantitative analysis of stone. In order to accomplish this purpose a newly developed isotachophoresis was applied for stone analysis.

MATERIALS AND METHODS

The isotachophoretic analyser used (Fig. 1) is the Shimazu IP-1B, equipped with a PGD-1 potential gradient detector and a recorder (Shimazu Seisakusho, Ltd., Kyoto, Japan). The principle of isotachophoresis is shown on Fig. 2. This is an electrophoretic analysis performed in the fluid phase. Leading electrolyte and terminal electrolyte are filled in reservoirs. The reservoirs contain respectively an anode and a cathode.

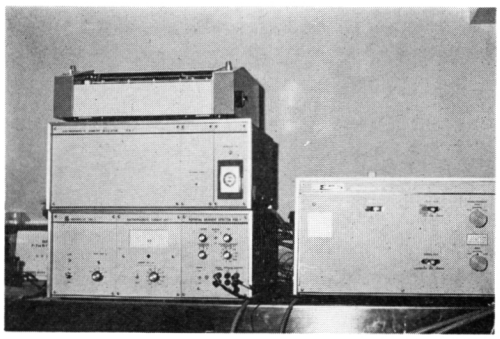

Fig. 1. The equipments.
Apparatus for isotachophoresis is placed on the right. The left middle is a current generator. The lowre is a potential gradient detector and the upper is a recorder.

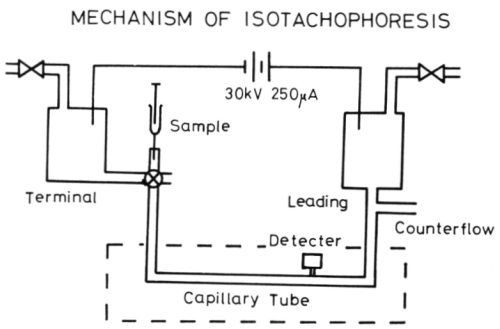

Fig. 2. Leading electrolyte and terminal electrolyte are filled in each reservoir. An electrophoretic capillary tube is connected between them. After the capillary tube is filled with leading electrolyte a sample solution is introduced. A steady current is supplied and the potential of ions is detected at the end of the tube.

An electrophoretic capillary tube is connected between the reservoirs. The tube is 0.5 mm in diameter and 20 cm in length and controlled at 20°C of the temperature. Microanalysis is one of the benefits of this method. Usually 10 μl of the sample is introduced into the sample tap with a microsyringe.

In our study 0.01 M histidine and 0.01M potassium acetate(pH 5.4) was used as the leading electrolyte and 0.01M tris acetate (pH 5.0) was used as the terminal electrolyte for the separation of cations. For the anions a mixture of 0.01M histidine and 0.01N histidine-HCl (pH 6.02) was used as the leading electrolyte and 0.01M caproic acid as the terminal electrolyte. Chemical agents for leading and terminal electrolytes, and all the standard materials were used in the highly purified form commercially available.

Stones were washed with water to remove blood and other adhering material. Dried and sectioned with a blade and powdered. One mg of the powder was dissolved with 10 ml of 0.01M HCl and 20–50 μl of the sample solution were applied to the analyser.

The electric current was stabilized at 75 μA for both cation analysis and anion analysis.

Ions were detected at the end of the electrophoretic capillary. They were identified by the ratio of potentials.

$$\text{potential ratio} = \frac{\text{terminal potential-potential of the sample ion}}{\text{terminal potential-leading potential}}$$

Zone length of ion correlates with the amount of the ion. As the ion moves in the same speed, the passing time of ion on the detector correlates with the amount of the ion.

RESULTS

Calcium, magnesium, oxalic acid, citric acid and phosphatic acid were satisfactorily separated and calculated. Potential gradient curves and standard curves were shown in Fig. 3 and Fig. 4 respectively. Satisfactory reproducibility was obtained. Linear relationship is observed between the passing time and the amount of ions introduced. Ammonium and carbonic ions, which are suggested as occasional components of urinary stones, escaped under the condition of the study. Uric acid moved more slowly than the terminal caproic acid and formed a round curve in the terminal ion.

The mobility of cystine and xanthine, very rare components of urinary stones, was smaller than that of uric acid and they could not be detected with the histidine-HCl and caproic acid system.

To identify and calculate the latter three substances 0.01M HCl adjusted to pH 8.5 with 2-amino-2-methyl-1, 3-proamediol was used as the leading electrolyte and 0.01M β-alanine adjusted to pH 10.8 with Ba $(OH)_2$ was, after filtration, used as the terminal electrolyte. Although the three

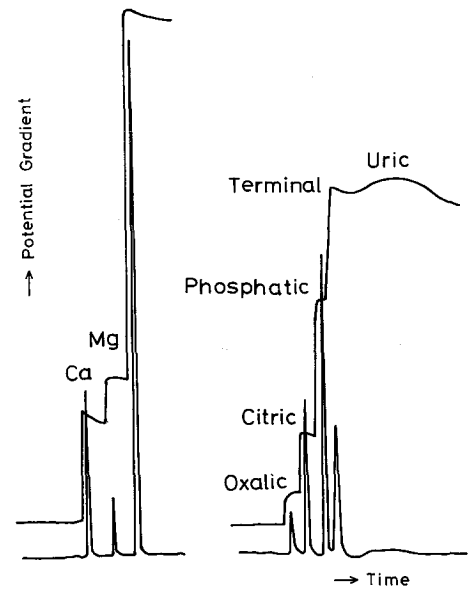

Fig. 3. Potential gradient curves of main urinary stone components. Integral and differential curves are shown.
Left: cation analysis
leading electrolyte: 0.01 M histidine and 0.01 M potassium acetate (pH 5.4)
terminal electrolyte: 0.01 M Tris acetate (pH 5.0)
Right: anion analysis
leading electrolyte: 0.01 M histidine and 0.01 M histidine-HCl (pH 6.02)
terminal electrolyte: 0.01 M caproic acid

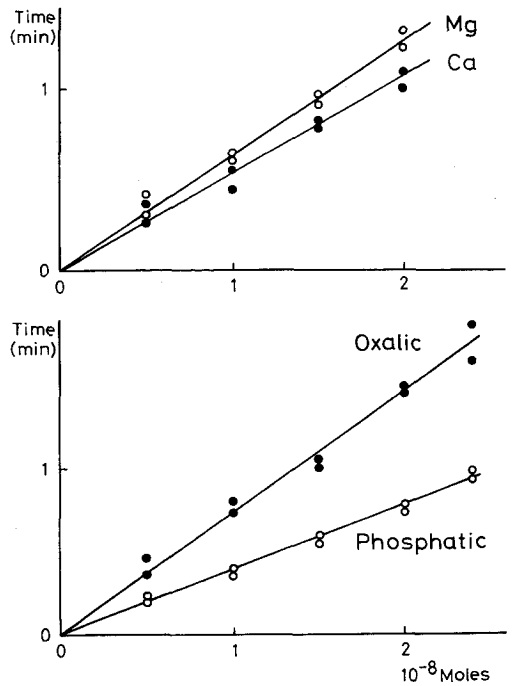

Fig. 4. Standard curves. The length of the zone, passing time in turn, well correlate with the amounts of ion.

substances could be separated and identified in the system, fast moving anions were not separated and they formed mixed zones.

We analyzed urinary stones with this method and the results obtained from the analysis of 58 stones were histogramatically shown in Figure 5.

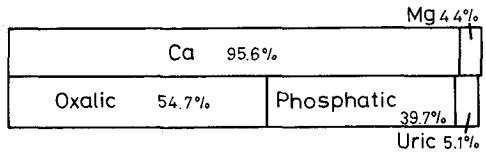

Fig. 5. Summary of analysis on 58 urinary stones. Mean value obtained from 58 urinary stones is shown.

DISCUSSION

The principle of isotachophoresis was analogued to displacement chromatogram[7]. In anion analysis, for example, all anions move towards the anode. In moving to the anode, the anions arrange themselves in order of mobility and once the arrange has been completed all the anions move in the same concentration and in the same speed[7]. The passage of each zone can be detected at the end of the capillary tube by their potential gradient, heat production or ultraviolet absorbance[1].

On the basis of our observation this method is useful for stone analysis because it is simple and rapid. The time required for a separation is about 20 minutes. It is well known that the composition of urinary stone differs in portion to portion. This method can deal with small specimens taken from any desired portion of the stone. When the stone is throughly powdered and mixed, mean value of the components can be easily obtained.

The electrolyte solution which completely satisfy our purpose has not been found. According to Beckers and associates[2] the step-height of uric acid is lower than that of cacodylic acid in using histidine-HCl as leading electrolyte and a thermometer as detector. The use of cacodylic acid for terminal electrolyte, however, failed to separate uric acid. Conversely, it was easily identified as a sloped peak in the terminal caproic acid. Caproic acid was therefore used as the terminal electrolyte in the anion analysis. Although the β-alanine-$Ba(OH)_2$ system can separate uric acid, cystine and xanthine between the boundry of the leading and terminal electrolyte, difficulties arise because of interactions of fast-moving ions. The system is therefore can not be recommended for the analysis of oxalic acid and phosphatic acid.

Chemical analysis of urinary tract stones (5, 6, 8) is not easy because each component must be estimated separately. An easy, simultaneous quantitative analytical method is desirable. It is the reason we tried to apply isotachophoretic method for urinary stone analysis. The method could not satisfy our purpose completely. All the possible components of urinary stones could not be detected. Ammonium and carbonate ions escaped from the detection. Stone constituents were not detected in their crystaline form. From these points isotachophoresis does not replace the existing methods.

However, it should be mentioned that

the analysis is performed in the fluid phase. It may be useful in experimental studies on the relationship between urinary constituents and precipitated stones such as crystallization from synthetic urine. Important constitutions of urinary stones can be simultaneously analysed.

It is found that the addition of a non-ionic detergent increases the sharpness of the the zone boundaries[1]. Further improvement of the present equipment would bring down an excellent tool for the study on urinary stone.

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(Accepted for publication, October 19, 1979)

和文抄録

イソタコフォレーシスによる尿路結石の分析

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イソタコフォレーシスを尿路結石の分析に応用することを試みた．

本法は液相で行なう電気泳動法で，イオンは泳動槽である細管内で移動度の順に並び，等濃度かつ等速で移動する．これを今回用いた材器（島津 IP-1B）の場合は電位差で検出する．つまり電位の高さによってイオンを同定し，検出器を通過する所要時間でイオン量を定量する．

本法は微量定量法であり，短時間に，簡単な手技で多種類の陽イオンまたは陰イオンを同時に分離定量できるが，尿路結石分析のための条件を全面的に満足するものではなかった．移動度の遅い尿酸，シスチン，キサンチンの分離定量には別の溶液を用いる必要があった．

しかしながら，液相で行なう短時間，同時定量法であるという特徴を生かせば，今後の尿路結石の研究に有力な武器になる可能性がある．