Could Infrared Spectroscopy Identify Melamine-Related Stone Using Melamine-Contained Mixture as A Reference?

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Background: Recently, a method of infrared spectroscopy analysis to identify melamine-contained stone was established by examining melamine powders mixed with true urinary stones. However, several studies demonstrated melamine could be interacted with cyanuric acid or uric acid in water through hydrogen bonds. It presents a hypothesis that the infrared spectrum of melamine-contained stone formed in urine is probably different from melamine-contained dry mixtures. This study is to testify is it true. **Methods:** The melamine-related mixtures were, respectively, prepared by mixing powders of melamine with cyanuric acid or uric acid in equimolar ratio. The melamine-related precipitates mimicking its related stone formation were, respectively, prepared by mixing melamine with cyanuric acid or uric acid in water at the given conditions. Subsequently, the

melamine-related mixtures and precipitates were analyzed by infrared spectroscopy. **Results:** The wave-number positions of powder mixtures of melamine–cyanuric acid and melamine–uric acid were a combination of these of their individual ingredients. The typical wave-number positions of melamine were showed in two melamine-contained mixtures. In contrast, these positions were disappeared or shifted greatly in the two melamine-related precipitates. In total, the spectrum of precipitates of melamine with cyanuric acid and uric acid had significantly differences with their powder mixtures. **Conclusions:** Our results indicate the identification of melamine-related stone by infrared spectroscopy could not use the infrared spectrum of melamine-contained mixtures as a reference. J. Clin. Lab. Anal. 27:59–61, 2013. © 2012 Wiley Periodicals, Inc.

Key words: infrared spectroscopy; melamine; cyanuric acid; uric acid; stone

INTRODUCTION

In the summer of 2008, an outbreak of kidney stones and renal failure in infants caused by the melamine-adulterated formula in China drew critical attentions to melamine toxicity for human (1). Analysis of melamine-related stones from infants by high-performance liquid chromatography showed the compositions were melamine and uric acid (2). In contrast, animal outbreaks of renal disease occurred worldwide in 2004 and 2007 were caused by con-ingesting melamine and cyanuric acid (3). No stone was observed but crystals of melamine cyanurate in renal tubules and collecting ducts were detected by infrared microspectroscopy (4, 5). The compositional analysis reveals melamine-related infant stones and animal crystals should be a respective result of the some sort of interaction of melamine with uric acid and cyanuric acid in urine. This supposition is also supported by several studies that demonstrate melamine could be

self-assembled and aggregated with uric acid or cyanuric acid to form lager clusters in water through N-H...O and N-H...N hydrogen bonds (Fig. 1A and B; 6, 7).

If it is so, it suggests the typical functional groups of melamine molecular such as NH₂ and C-N will be affected, the infrared spectrum of melamine–uric acid stone or melamine–cyanuric acid crystal should be distinguished from these of powder mixtures of melamine with uric acid or cyanuric acid because infrared spectroscopy is based on the interaction between the infrared light and the bonds and functional groups of compounds. To testify this hypothesis, the precipitates of melamine–uric acid

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Fig. 1. The hydrogen-bonded chemical structure of melamine-related precipitates, and the infrared spectrum of melamine-related powder mixtures and precipitates. (A) melamine-cyanuric acid, (B) melamine-uric acid, (C) melamine-cyanuric acid powder mixture, (D) melamine-uric acid powder mixture, (E) melamine-cyanuric acid precipitate, (F) melamine-uric acid precipitate.

and melamine-cyanuric acid were prepared. Our “in vitro” studies demonstrated at pH 5.5, the melamine and uric acid at soluble concentrations in water could form precipitates with special appearances. While melamine and cyanuric acid at very low concentrations and every physiological urinary pH in water could easily form precipitates with another appearances. Figure 1C and D showed

the infrared spectrum of powder mixtures of melamine-cyanuric acid and melamine-uric acid at a molar ratio of 1:1. The wave-number positions of each mixture were a combination of these of their individual ingredients. Take the wave-number positions of melamine for example, the presence of melamine was shown by absorptions at 3,470, 3,420, 3,335, 3,215, 1,653, 1,555, 1,470, 1,440,

1,030, and 815 cm^{-1} as the data of Chen et al (8). These melamine wave-number positions were revealed in our two melamine-contained mixtures. However, in contrast, they were disappeared or shifted greatly in the two melamine-related precipitates as shown by Figure 1E and F. This phenomenon is in agreement with the previous hypothesis.

The results in our study indicate the identification of melamine-related stone by infrared spectroscopy could not use the infrared spectrum of melamine-contained mixtures as a reference. In a previous study, Grases et al. had demonstrated infrared spectrum of the “in vitro” formed melamine-uric acid crystals was identical to the infant’s melamine-related stone (9). Therefore, the study design by Chen et al. who established a reference of infrared spectroscopy analysis to identify melamine-contained stone by examining melamine powders mixed with true urinary stones (8) are not rational. Readers and analysts of stone compositions should be alerted when they use infrared spectroscopy to identify melamine-related stone. Recently Liu et al. revealed low-dose melamine played one more culprit in calcium stone in Taiwanese adults (10). It suggests a timely and correct technique detecting melamine-related stone is still required. To the fast and convenient infrared spectroscopy well used for melamine-related stones, we need more works to do.

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