Urol Res (1999) 27:135–140
© Springer-Verlag 1999

ORIGINAL PAPER

Daniel Bongartz · Anke Schneider · Albrecht Hesse

Direct measurement of calcium oxalate nucleation with a laser probe

Received: 19 March 1998 / Accepted: 9 July 1998

Abstract To understand the genesis of urinary stones different crystallization models have been developed to simulate the crystallization processes that occur at the beginning of the stone formation. In this study a laser probe working on the principle of the measurement of back-scattered light was mounted on a batch crystallization model, in which calcium oxalate nucleation was induced by titration of an artificial urine with ammonium oxalate. With the laser probe the particle numbers and particle size distributions in the range from 1–250 μm can be measured in a single experiment. Measurement is performed directly at the place of crystal development and, therefore, falsification of the results (by dilution, mechanical transport or isolation, temperature gap, multiple measurements, mathematical calculations after indirect measurements, etc.) is prevented. In a basic urine, containing 7.5 mmol/l calcium (4.13 mmol/l free calcium), nucleation of calcium oxalate appears at a concentration of 0.58 mmol/l oxalate. At the start of nucleation the particle size rises to 7 μm. After addition of citrate and magnesium in concentrations up to 12 mmol/l the metastable limit is clearly shifted up to 1.50 mmol/l oxalate. Both findings are in good agreement with the literature. The laser crystallization model has proved to be useful for the testing of inhibitors and promoters of urinary stone formation.

Key words Calcium oxalate · Nucleation · Crystallization model · Particle size distribution · Laser probe · Inhibitors

D. Bongartz · A. Schneider · A. Hesse
Division of Experimental Urology,
Department of Urology,
University of Bonn,
D-53105 Bonn, Germany

A. Hesse (✉)
Experimentelle Urologie,
Sigmund Freud Strasse 25,
D-53015 Bonn, Germany

Introduction

The initial step in the formation of urinary stones is the nucleation as a result of supersaturation of urinary components. These initial microcrystals start to grow or agglomerate and eventually form the pathological urinary stone. The extent of nucleation, crystal growth, and agglomeration is mainly determined by urinary chemical thermodynamic and kinetic factors such as ion-active production and high or lower molecular inhibition. Therefore, specific crystallization risk indices such as the relative supersaturation [6] or the Tiselius risk index [18] have been derived semi-empirically from the measurement of urinary parameters such as calcium, oxalate, citrate, or uric acid. However, due to the complex interaction of the urinary components the indices are only approximations of the real crystallization properties of the urine [12]. Furthermore, the influence of macromolecular inhibitors such as Tamm-Horsfall protein or glycosaminoglycans, or the initiation of the crystallization process by seed crystals, is not considered in these analytical-mathematical methods.

Different batch and flow crystallization models, in which the stone-forming processes of nucleation, agglomeration, and growth are simulated and the actual crystallization (i.e., irrespective of urine composition) is measured, have been developed [1, 4, 16, 17]. The simulation of the crystallization processes in these models is well established and reproducible, whereas the measurement of the crystallization is still problematic for several reasons. A detection system for crystal sizes and concentrations has to fulfill some specific requirements:

- the determination must be independent of crystal and matrix concentration;
- small and large particles ranging from 1 to about 300 μm have to be recorded simultaneously;
- the detection system must not affect the actual crystallization process (e.g., by thermostatic gap, mechanical forces, or surface activity);

– the particle shape has to be taken into consideration;
– the measurement has to be made in (quasi) real-time.

The most common indirect method for the determination of crystal concentration is the measurement of the turbidity of the solution by a photometer [9]. For the determination of particle size distributions (PSD; histogram of particle size and frequency) the Coulter counter, which measures the volume displaced by the particles, is widely used [15]. The measurement of laser light scattering and intensity can also be used for the determination of PSD [2]. The reference method (DIN 66 111 [13]) for the measurement of PSD is the measurement of particle sedimentation speed in a viscous fluid, which is not applicable to (on-line) measurement in urine. In particular the fast and reliable Coulter technique and the laser scattering method are limited by matrix concentration and particle size range.

In this paper we present the application of a total reflection laser probe which, in combination with a batch crystallization model, allows the on-line measurement of PSD in a wide range of particle sizes even at high particle concentrations.

Materials and methods

The laser probe (Messtechnik Schwartz, Duesseldorf, Germany) consists of a stainless steel tube with a diameter of about 40 mm (Fig. 1). In the tube a rotating optical system with different optical lenses and prisms is mounted. The laser source is coupled to the optical system by a fibreglass wire. In the rotating optics the laser beam is deflected from the center axis, so that it describes a scan circle in the crystallization medium. The focus point, which has to be optimized with respect to the crystal concentration, can be adjusted by moving the rotating optics back and forth. If the laser beam hits a crystal, it is reflected partially through the probe and can be detected by a photodetector. The rough detector signal is processed by an electronic device, which can be adapted in threshold and slope to the specific reflection properties of every crystal type, and finally the signal is evaluated by the system software. Each signal represents a single crystal (i.e., a count). The crystal size can be calculated from the known angular speed of the rotating optical system and the duration of the signal. The geometrical shapes of different crystal types are included in the calculation by means of form factors.

The laser probe is attached to a batch crystallization model (Fig. 2) [3]. The model consists of a glass vessel at 37°C in which calcium oxalate (CaOx) nucleation is initiated by titration of an artificial urine [7] with ammonium oxalate [$(NH_4)_2Ox$] by an automated titration station. The course of the crystallization process is evaluated with the laser probe by monitoring the relative count rate (i.e., crystal number registered by a photodiode of each reflected laser impulse according to the time base of the measurement of the scanning laser probe) and the PSD during the titration. The basic parameters of the model and the laser probe are listed in Table 1. The system parameters used here (i.e., continued titration with oxalate for maintaining CaOx supersaturation) are applied for measuring the metastable limit of CaOx with respect to crystallization inhibitors. This makes up only a small part of the system's abilities: with its automated titration stations it is capable of adjusting and maintaining different saturation states in the crystallization vessel (e.g., adjusting metastable CaOx concentrations to observe crystal growth and aggregation or to estimate nucleation rates).

To evaluate whether the laser probe achieves results comparable to those obtained by other methods we tested the effect of the two well-known crystallization inhibitors – citrate and magnesium – on the metastable limit of CaOx by adding them in concentrations up to 12 mmol/l to the basic artificial urine before titration. The experiment was performed 4 times for each concentration. To obtain a general idea of the change in the PSD the results are expressed as a single parameter: the particle size d(50). The d(50) value represents (similar to the median in a Gaussian distribution) the central value in a particle size distribution (see DIN 66141 [13]). For example, a particle size d(50) of 5 μm means that 50% of all particles are equal to or smaller than 5 μm.

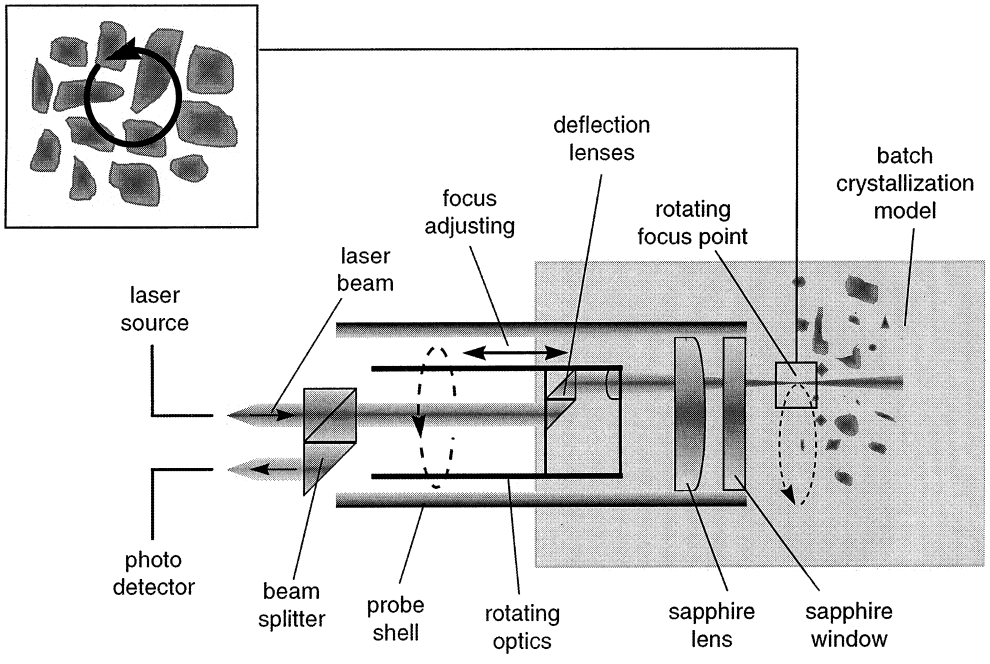

Fig. 1 Diagram of the construction of the laser probe. Upper left: scan circle of the laser in the focal plane with scanned crystals

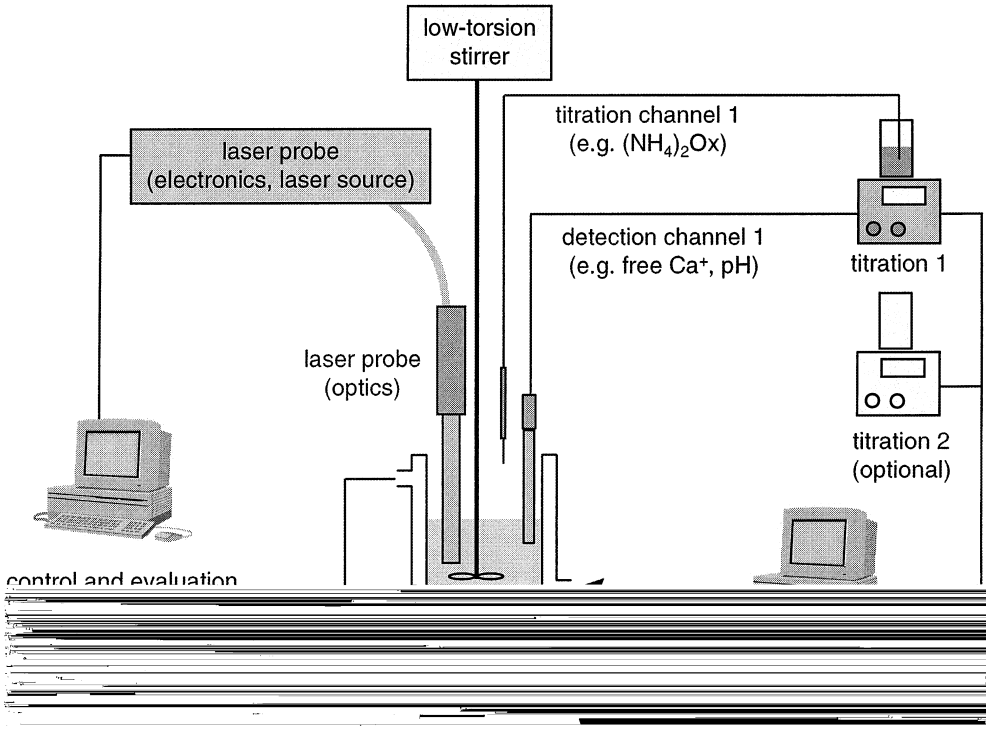

Fig. 2 Diagram of the laser probe connected to the crystallization model

Table 1 Basic parameters of the crystallization model and the laser probe [$(NH_4)_2Ox$ ammonium oxalate]

Parameter	Value
Volume of artificial urine	200 ml at starting time
pH of artificial urine	6.0 at starting time
Total calcium concentration	7.5 mmol/l
Concentration of $(NH_4)_2Ox$	20 mmol/l
Titration speed of $(NH_4)_2Ox$	1 ml/min
Total $(NH_4)_2Ox$ added	40 ml
Stirrer speed	330 rpm
Measurement time	8 s

Results

Due to background noise and the fact that the measurement is near to the physical resolution of the laser probe, a count rate of about 75 and a particle size d(50) of about 2.5 μm were observed in the basic artificial urine before starting the titration. In the basic urine without addition of promoters or inhibitors the concentration of free calcium was 4.13 mmol/l as determined by a Ca-sensitive electrode. After starting the titration we eventually observed a simultaneous increase in both the relative count rate and the particle size d(50), after addition of 6.75 ml ammonium oxalate to the basic artificial urine (Fig. 3). This represents an oxalate concentration of 0.58 mmol/l. The d(50) size increased to about 7 μm. No further change in the PSD was observed after continued addition of oxalate to a final concentration of 3.3 mmol/l total oxalate.

After the addition of 6 mmol/l citrate to the basic urine the start of nucleation is considerably delayed by a factor of about 2, and the relative count rate at the end of titration is about 33% lower than in the urine without additives (Fig. 4). The delay in the start of the nucleation shows a clear correlation with the concentration of citrate (Fig. 5, left). The free calcium at the start of the titration decreases from 4.13 mmol/l in the artificial urine without additives to 0.53 mmol/l in the urine containing 12 mmol/l citrate (Fig. 5, right). The experiments were checked for reproducibility within a series and from day to day. We could not find any difference in the PSD between the individual measurements. Additionally, particle sizes were cross-checked by light microscopy.

With regard to the shift in the metastable limit the results for magnesium are similar to those obtained for citrate. We observed a considerable delay in nucleation, which is correlated with the concentration of magnesium (Fig. 5, left). In contrast to the situation with citrate, this cannot be caused by a decrease in free calcium, because it is almost constant for every magnesium concentration (Fig. 5, right). Additionally, the relative count rate did not noticeably increase after addition of magnesium. As for citrate, the particle size d(50) after nucleation was about 7 μm.

Discussion

The system described is suitable for the measurement of crystallization processes in many respects. Due to its technical principle the laser probe is capable of measuring the PSD directly at the place the crystals are developing. Neither mechanical transportation to a

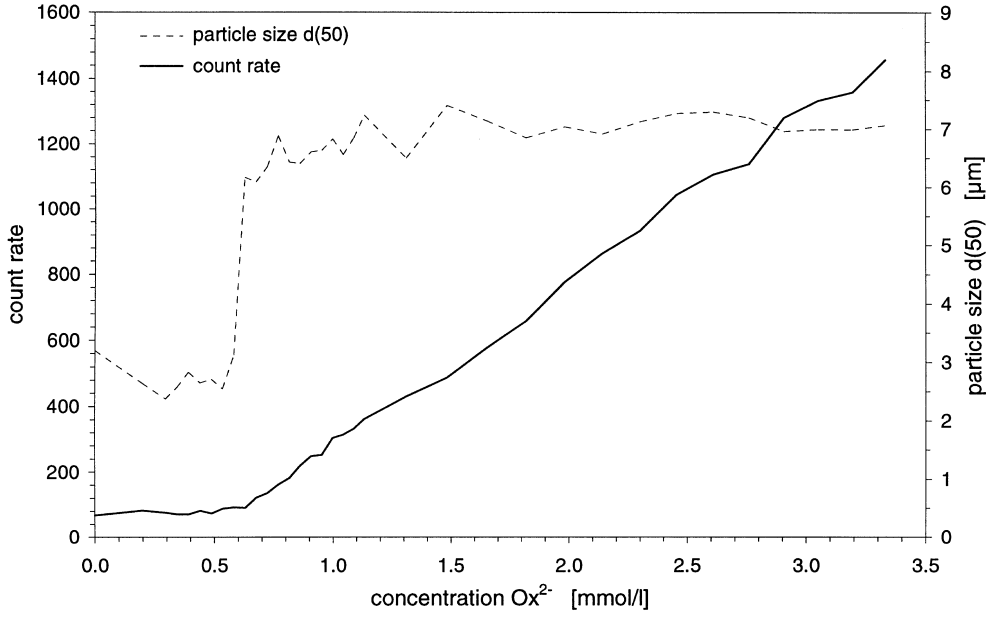

Fig. 3 Course of count rate and particle size d(50) in artificial urine without the addition of promoters or inhibitors during titration with ammonium oxalate

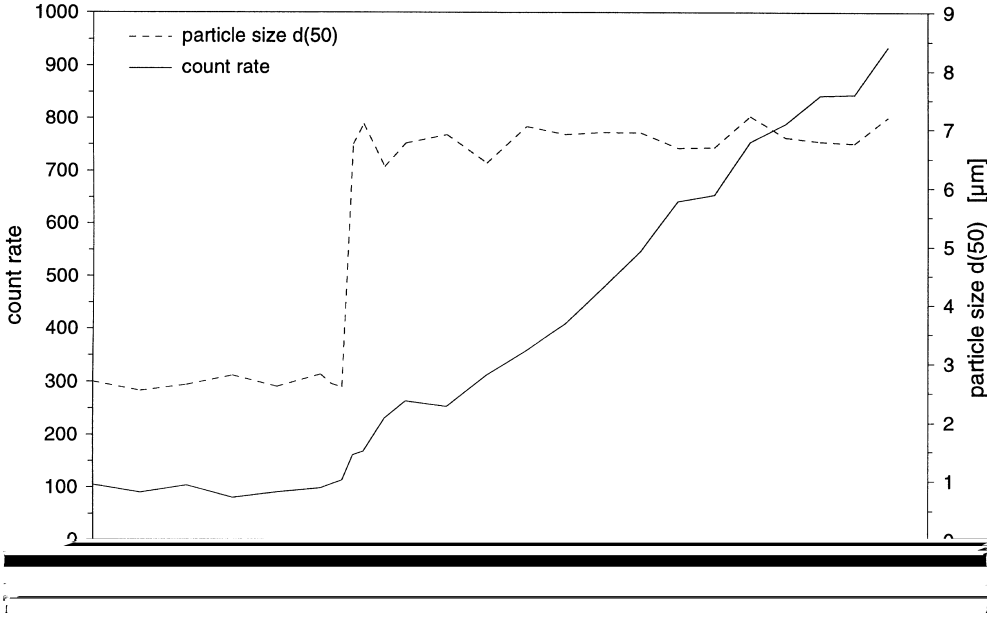

Fig. 4 Course of count rate and particle size d(50) in artificial urine containing 6 mmol/l citrate during titration with ammonium oxalate

measuring device with possible damage of the crystals [14] nor any filtration or isolation steps [5] are required for the determination of the PSD. Because the laser probe is an integral part of the crystallization vessel the determination is easily carried out in the original crystallization medium at the desired crystallization temperature. The measurement of the backscattered laser light requires little penetration of the laser beam into the crystallization medium (the focus of the laser is typically adjusted to a distance up to 1mm from the sapphire window: see Fig. 1), so that particle concentrations up to 80% (volume) can be measured with appropriate adjustment of the electronic device. By applying a low-torsion nonmagnetic-driven stirrer no friction forces are introduced to the crystals at the bottom of the crystallization vessel.

The possibility of the simultaneous real-time determination of the relative number of crystals and the PSD in a single experiment is a main advantage of the system. In contrast to the Coulter counter or laser scatter systems the PSD can be determined even at high crystal concentrations in a range from about 1 to 250 µm without a change in the measuring device (i.e., capillaries or focusing lenses).

In the system described, CaOx nucleation is induced in artificial urine by a continuous increase in the oxalate concentration. At an oxalate concentration of 0.65 mmol/l we observed a simultaneous increase in