A variety of equipment is used for the observation of precipitation processes which occur in urinary samples. The Bonn-Risk-Index, a measure of the calcium oxalate crystallization risk of human urine, has been developed with the use of an in-line laser-probe gauge. For basic research or in clinical laboratories, however, this instrument, which fulfills industrial requirements for the evaluation of particle size distributions, is not widely available. The evaluation of an alternative method to determine the Bonn-Risk-Index based on a more commonly available apparatus would therefore be useful.

In vitro crystallization experiments with 124 native urine samples from stone-forming and non-stone forming individuals were performed in order to determine their crystallization risk according to the Bonn-Risk-Index approach. The onset of an induced urinary crystallization was detected by simultaneous sample monitoring with an in-line laser-probe and a conventional dip-in photometer. A decrease of the sample's relative light transmissivity from initially 100% to 98% was assumed to be a reliable photometer-based criterion to indicate that crystallization actually began. The laser-probe signal was set as the reference measure.

Linear regression analysis of the results of the laser-probe and the photometer-based Bonn-Risk-Index determinations reveals a significant and close correlation between the two measures. Method comparison by statistical evaluation shows i) that no significant deviation from linearity exists and ii) that both methods are statistically identical. The differences in the results are small enough to be confident that the photometer can be used in place of the laser-probe for clinical purposes.

The photometer is a reliable, easy-to-use and cost-effective method for the determination of a triggered crystallization event in a urine sample. The assumed 98% criterion allows the determination of the Bonn-Risk-Index with adequate accuracy.
were collected from healthy volunteers and from CaOx stone-formers. During the collection period, urine was stored at +4°C to maintain original conditions. Crystallization experiments to test the urines for their CaOx formation risk according to the BRI method were carried out immediately after collection as described in detail before (2).

The initial urinary concentration of ionized calcium ([Ca\(^{2+}\)]) was measured by a calcium-selective electrode (Metrohm AG, Herisau, Switzerland). Precipitation of CaOx in a 200 ml aliquot of the urine sample was triggered by step-by-step addition of ammonium oxalate solution (0.04 N, 0.5 ml/step, 1.5 ml/min). The quotient of [Ca\(^{2+}\)] and the amount of ammonium oxalate (Ox\(^{2–}\)) added until precipitation was detected is termed the "Bonn-Risk-Index", BRI=[Ca\(^{2+}\)]/(Ox\(^{2–}\), [per liter].

**Apparatus**

Figure 1 illustrates the experimental setup to examine the moment of crystallization of a urine sample simultaneously with an *in situ* laser-probe crystal system analyzer (ORM 3D, Meßtechnik Schwartz GmbH, Düsseldorf, Germany) and a dip-in photometer (Photometer 662, Metrohm AG, Herisau, Switzerland), operating at a wavelength of 620 nm. The assays were performed in a water-jacketed glass vessel maintained at 37°C.

The laser-probe and the dip-in photometer were placed in such a way that the urine and the suspended particles could flow laminarly, directly, and successively through the measurement zones of both instruments. The laser beam and the photometer light were perpendicularly oriented with respect to the measurement zones’ flow field (Figure 1).

The laser-probe analyzes the number and the individual dimensions of suspended particles with sphere-equivalent chord lengths between 0.5 µm and 250 µm. Particles floating into the detection volume are recorded by a horizontally and highly frequent orbiting laser beam focus. A detector device detects the back-scattered light and generates an electrical signal dependent on the duration of the back-scattered light flash. From these data, a count rate and various parameters describing particle size distribution (PSD) can be computed (6). In the case of particle formation, the count rate and the characteristic parameters of a PSD will change.

In contrast, the photometer simply measures the sample’s relative light transmissivity. Particle formation and alteration are related to a non-particle size-specific decrease in the light transmissivity. From the experience of a number of pilot studies, a decrease in the relative light transmissivity from initially 100% to 98% is assumed to be a definite criterion of the existence of an ongoing particle alteration process which can be interpreted within the experimental setup as a result of CaOx precipitation. Whereas the light transmissivity decreases slowly at first, with an increasing number of titration steps from 100% to approximately 98%, the rate of decrease of this parameter increases rapidly after passing the 98% limit without further titration. The higher the sample’s BRI, the more rapid the decrease.

No variability of the light transmissivity was observed, measured on the same urine at the same time but without adding ammonium oxalate. Figure 2 illustrates the laser-probe and photometer recordings.

**Statistical analysis**

As the value of the BRI is inversely related to the amount of added ammonium oxalate, the crystallization risk according to the BRI increases in increments of e.g. 0.5 l–1, 1.0 l–1, 2.0 l–1, 4.0 l–1. Therefore, the laser-probe and photometer-based strategy of the BRI determination is identical when the ratio of BRI\(_{\text{Laser}}\)/BRI\(_{\text{Photo}}\)=1. Thus, the difference log BRI\(_{\text{Laser}}\)–log BRI\(_{\text{Photo}}\)=0. In a log BRI\(_{\text{Laser}}\) vs. BRI\(_{\text{Photo}}\) plot, the linear regression line shows a gradient of 1 when both methods reveal identical results.

Mean values and standard deviations (SD) were computed. Both methods were tested for equality according to the methods of Passing and Bablok (7) and Bland and Altman (8).

**Costs**

The approximate instrument costs for the laser-probe and the photometer, both of the latest generation, were calculated according to the manufacturer’s information; they amount to approximately US$ 45,000.00 and US$ 4,200.00, respectively. In both cases, a standard PC for data storage and computing is required. Furthermore, a computer-controlled titration unit, as well as a calcium-selective electrode are required. The costs of reagents (ammonium oxalate, standard solutions for ion-selective electrodes) and glassware are negligible.

---

**Figure 1** Experimental setup for synchronous determination of the onset of urinary calcium oxalate crystallization via laser-probe and photometer.
Results

BRI values between 0.04 l⁻¹ and 32.94 l⁻¹ were determined. This range covers the wide variety of normal and pathological conditions of CaOx crystallization in human urine. The mean BRI_{laser} value and the median BRI_{laser} value amount to 2.81 l⁻¹ and 1.33 l⁻¹, respectively.

Correlation of methods

In Figure 3 the results of the laser-probe and photometer measurements are compared in a logBRI_{laser} vs. logBRI_{photo} plot.

Linear regression analysis (y=0.9856x-0.0006) revealed a significant (p<0.001) correlation coefficient of r=0.997. Method comparison according to Passing and Bablok (7) revealed i) that no significant deviation of linearity exists and ii) that both methods are identical.

In Figure 4 the variation of the BRI_{laser}/BRI_{photo}-ratio on the logBRI_{photo} is shown. Furthermore, the “limits of agreement” as mean ratio ± 2 SD are given. This plot is essentially the same as the Bland-Altman plot (8), however, for clearer interpretation, we draw on the x-axis the value of logBRI_{photo} instead of the (geometric) mean of BRI_{photo} and BRI_{laser}.

Figure 3  Log-log scatter plot of laser-probe vs. dip-in photometer based BRI values obtained from the investigation of 124 urine samples. It is assumed that a decrease in the urine’s relative light transmissivity from initially 100% to 98% marks the moment of detection of crystallization in the photometer experiments. According to the regression analysis of Passing and Bablok (7) both methods are identical.
The highest ratio of BRI\textsubscript{laser}/BRI\textsubscript{photo} amounted to 1.499 at BRI\textsubscript{photo}=17.87 l–1, the lowest determined ratio was 0.722 at BRI\textsubscript{photo}=2.604 l –1 (Figure 4). The mean BRI\textsubscript{laser}/BRI\textsubscript{photo} ratio amounted to 1.009; a related SD of 0.107 was computed. No relevant correlation between BRI\textsubscript{photo} and the BRI\textsubscript{laser}/BRI\textsubscript{photo} ratio was observed. All data are consistent with a Gaussian distribution with a SD of 0.107. Outliers were not encountered.

Discussion

At a time when a CaOx crystallization event in urine samples is clearly observed, a number of processes had already taken place. However, these early processes are beyond experimental control as detection limits restrict the possibilities of a direct and unequivocal observation. Therefore, the determination of the “onset” of crystallization is not the observation of a process which is just beginning but rather it is “gaining insight” into ongoing alteration of already formed crystals.

The changes in PSD due to ongoing particle size alteration caused by thermodynamic non-steady state conditions, for example, are not reflected in an unequivocal signal pattern. Therefore, empirically derived break-off criteria must be set which would allow a reliable confirmation that a crystallization process has occurred.

The laser-probe principle allows the statistical computation of a number of PSD parameters which are based on individual counting and direct size measurement of suspended particles as detected during a measurement cycle (i.e. over a certain time span). Due to the possibility of almost real-time observation of the time-dependent evolution of various PSD parameters, the break-off point for “real” detection of crystal formation is indicated by the (simultaneous) change of derived parameters most sensitive in crystal formation. From our experience, these parameters are: i) the count rate, ii) the median of the number-weighted PSD (c50[0]), iii) the median of the surface-weighted PSD (c50[2]) and iv) the percentage of particles larger or equal to a certain particle size (R(X)[0]). The variable X in the latter parameter is set to be 14 µm, according to the smallest lumen diameter of the smallest renal tubule segments, the descending and ascending limb (9). Even under difficult experimental conditions, for example with small amount of precipitate or slow rate of salting out, at least one of these parameters would result in a signal sufficient for reliable interpretation.

The photometric principle, in contrast, only allows the detection of an integral change of the sample’s relative light transmissivity due to a change in the sample’s turbidity. The reasons for such a change cannot be evaluated: for example, in case of crystallization of the same amount of CaOx, the sample which formed many of small particles shows a higher degree of light transmissivity reduction than the sample which formed larger but fewer particles. This makes definition of a break-off criterion more difficult.

However, although both methods of investigation measure quite different physical parameters, their results with respect to (early) determination of an ongoing crystallization process can be compared with reliable accuracy. Using the 98% break-off criterion for the photometer, it is possible to predict values of BRI\textsubscript{photo} with a mean inaccuracy of (i.e. SD) 11% from BRI\textsubscript{laser} data. This inaccuracy is in the order of magnitude which is also found in other methods of crystallization risk evaluation, for example the risk evaluation by computation of the urinary supersaturation from a bio-
chemical analysis (10). Due to the incremental addition of ammonium oxalate to the urine sample, the similarity of the results obtained using the two instruments is slightly better when the urine under study has a low BRI as opposed to a high one. However, the limits of agreement for the $BRI_{\text{laser}}/BRI_{\text{photo}}$ ratio (1.22 and 0.78) are small enough to be confident that the photometer can be used in place of the laser-probe for clinical purposes over the whole range of BRI.

From the observation of SD=0.11 it can also be concluded that the reproducibility of both methods of measurement is high. Assuming that both methods show the same statistical fluctuations, the results of each method obtained during repeated measurements on the same subject vary by $100(SD/2^{0.5})=7.78\%$. With respect to the BRI approach, this variation is not clinically important.

The laser-probe is an ideal instrument which fulfills basic research requirements. A number of statistical PSD parameters derived from single-particle size measurements can be obtained with it. The moment of a clear crystallization can be easily determined as the initial PSD parameters change systematically during ongoing crystallization. This process can be unequivocally detected by the laser-probe. Post-crystallization processes which (may) alter the size distribution of the suspended crystals can also be reliably investigated (6).

Metaphylactic measures by, for example, dietary measures (11), are necessary to reduce the recurrence rate of CaOx crystal formation (12). A regular monitoring of the CaOx crystallization risk of a stone-forming person is strongly recommended in order to obtain information about the effectiveness of the treatment. The determination of the BRI is a suitable, fast and easy-to-perform method for such a screening. Depending on the value of the observed risk, the metaphylactic treatment can be adapted to the actual risk situation. This strategy optimizes the patient's treatment by a more individual approach. This may reduce expensive overtreatment. The photometer is an ideal instrument for the routine determination of BRI as it provides an adequate accuracy, is easy to use and cost-effective.

Acknowledgements

This study was supported by the Deutsche Forschungsgemeinschaft (DFG) (Grant He-1132/11–4). The authors gratefully acknowledge the valuable suggestions given by Prof. Siekmann, Department of Clinical Chemistry, University of Bonn, which have improved the manuscript. We also thank Mrs. Dentler who provided editorial help.

References


Received 19 February 2002, revised 19 April 2002, accepted 25 April 2002

Corresponding author: Norbert Laube, Ph.D., Klinik und Poliklinik für Urologie, Experimentelle Urologie, Sigmund-Freud-Strasse 25, 53105 Bonn, Germany Phone: +49 (0) 228 287 9106, Fax: +49 (0) 228 287 6344 E-mail: norbert.laube@ukb.uni-bonn.de