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IN VITRO STUDIES OF ENCRUSTATIONS ON CATHETERS, A MODEL OF INFECTION STONE FORMATION

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Abstract

Deposition of infection-induced stone material on urinary catheters is a common problem in urological patients. Therefore, a crystallization model was developed in order to form this material in a reproducible manner. Furthermore, the dissolving potency of two solutions (Suby-G[®] and Solution-R[®]) was investigated with this model. The encrustations were examined by infrared (IR) spectroscopy, chemical encrustation analysis, scanning electron microscopy (SEM) and X-ray microanalysis. In addition, the encrustations were calculated from the results of the urine analysis, before and after each experiment, as contents remaining in each artificial bladder. The model conditions changed during the experiment and led to supersaturation for struvite. The encrustation analysis resulted in 73.5% (IR spectroscopy) and 78.5% struvite (chemical encrustation analysis) respectively. The calculated encrustation by urinary losses of stone forming contents resulted in 79.3% struvite. SEM showed that after the experiment, the catheters carried large amounts of mineral deposition and bacterial biofilm on their inner and outer surface. During the experiment, the catheters lost their homogeneous surface; cracks appeared and material was lost. X-ray microanalysis showed peaks for Mg, P and Ca (infection-induced stone material), Si (catheter material), Al (sample carrier) and Ag (conductive material) and supported the encrustation analysis. The solutions tested showed high dissolving capacities for infection stone material. After irrigation of the bladder with both solutions tested, 70% and 85% respectively, of struvite was dissolved.

Key Words: Urinary tract infection, struvite, catheter encrustation, synthetic urine, infrared stone analysis, chemical encrustation analysis, catheter irrigation, scanning electron microscopy, X-ray microanalysis.

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Introduction

Urinary tract infections are the most frequent nosocomial infections and they are often connected with, or caused by catheterization [15, 25, 35]. Formation of infection-induced stone material on catheters and in the bladder is a common complication [2, 4, 6]. The encrustations consist of struvite (magnesium ammonium phosphate hexahydrate, MAP) and calcium phosphate (carbonate apatite, CaP), encouraged by alkaline conditions [8, 29]. The alkaline milieu is caused by infection with urease-producing bacteria (e.g., *Proteus mirabilis*) [16, 18]. The changes in physicochemical conditions, rising pH-value and hyperammoniuria lead to supersaturation for struvite and carbonate apatite [13]. Encrustations inside the catheter can obstruct the drainage (catheter eye and lumen) and force a new catheterization with additional infection risk. Encrustations outside the catheter can damage the tissue and thereby lead to further complications, such as strictures of the urinary tract [9]. Remaining crystals in the bladder favor stone aggregation and growth, and often cause reinfections. Additional investigations have examined conditions of stone formation [12, 17, 20, 30], possibilities to decrease infection risk [1], to eliminate urinary tract infection [22, 27], to prevent stone formation [14, 23, 31], to minimize tissue injury [3, 8], to prevent a catheter's functional disorder [21] and to dissolve already existing infection stone material [5, 7, 8, 24, 26, 28, 32, 34].

Materials and Methods

A model has been established with six glass bladders (double walled glass vessels, capacity 250 ml) connected in parallel. The system (the urine vessel and the bladder) was kept at 37°C by a thermostatic water circulation. Sterilized synthetic urine (filtered through a 0.22 µm filter), composed according to Griffith [13] (Table 1) and supplemented with 15 g/l trypticase soy broth (soybean casein digest; sterilized by autoclavation) was pumped into each bladder at a rate of 81 ml/h and was collected separately after single bladder passage. Each bladder had a separate urine supply. The bladders were inoculated (by adding 6 ml of a dextrose nutrient

Table 1: Composition of the synthetic urine used (according to Griffith [13]), completed with a nutrient solution.

Contents	mmol/l
Calcium	4.43
Magnesium	3.25
Phosphate	23.57
Citric acid	2.26
Oxalic acid	0.15
Sodium	132.03
Potassium	64.52
Ammonium	18.52
Chloride	146.77
Sulfate	16.05
Creatinine	9.72
<hr/>	
Trypticase soy broth with ...	g/l
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Casein	8.5
Soybean meal	1.5
Sodium Chloride	2.5
Dipotassium Phosphate	1.25
Dextrose	1.25

solution) with a *Proteus mirabilis* strain (strain No. 01592/91) at a bacterial count of 10^7 colony forming units per milliliter (c.f.u./ml).

Silicone coated (inner and outer surface) latex catheters (Hoyer Nelathon[®]; size: 18 Charrier, filled with 10 ml) were installed into a silicone rubber plug closing the bottom of the bladders. The residual urine volume was 22.1 ml per bladder. Each experiment ran for 30 hours. Ten different conditions were used in each bladder, so that in all, sixty experiments could be evaluated in this study (Figure 1). All calculations and statistics were established by BMDP-software programs [RHRZ-Bonn, descriptive statistics: mean or average (avg.), standard deviation (SD), coefficient of variation (CV); significance tests: (a) independent variables, Wilcoxon paired rank test, (b) groups within a variable, Kruskal rank test].

To describe microbial activity, bacterial concentration (c.f.u./ml; Urotube Hoffmann LaRoche[®]) in three dilution levels (start: 10^2 , 10^3 , 10^4 end: 10^4 , 10^5 , 10^6 c.f.u./ml), ammonium concentration (potentiometry) and pH-value (potentiometry) was measured at the beginning and the end of each experiment.

The urine was analyzed for magnesium, calcium (atomic absorption spectroscopy, AAS), inorganic phosphate (molybdenum blue, spectrophotometry, MerkoTest 3331[®]) and oxalic acid (ion chromatography, IC) to calculate the quantity of urine contents remaining in each bladder system as encrustation material. Furthermore, urine was analyzed for potassium, sodium (flame photometry), chloride (coulometry), inorganic sulfate (nephelometry) and citric acid (enzymatic, citrate lyase) to calculate the relative supersaturation of stone materials

Table 2: Composition of the tested citric acid containing solutions (Suby-G and Solution-R).

Contents	Suby-G (mmol/l)	Solution-R (mmol/l)
citric acid	198	303
magnesium	94	332
EDTA	0.34	0.34
pH-value	4.1	3.8

(struvite and apatite) by the EQUIL-computer program [10, 11].

Infrared (IR) spectroscopy analysis of encrustation material was carried out for all involved model surfaces and for the whole bladder system [19]. Infection stone material was quantified for each involved model surface by chemical encrustation analysis using their magnesium, calcium, phosphate and oxalic acid contents, determined after dissolution with hydrochloric acid (1 N HCl for 1 hour). Struvite was calculated as magnesium ammonium phosphate hexahydrate (MAP; $MgNH_4(PO_4) \cdot 6H_2O$; 245 g/mol) by magnesium, calcium oxalate as calcium oxalate monohydrate (COM; $CaC_2O_4 \cdot H_2O$; 146 g/mol) by oxalic acid and calcium phosphate as carbonate apatite $\{Ca_{10}(PO_4,CO_3)_5(OH,CO_3)_2\}$; 1460 g/mol) by calcium, reduced for the calcium already bound in calcium oxalate [30]. Additionally the encrustations were calculated by urinary losses of magnesium, calcium, phosphate and oxalic acid for each bladder (using the same chemical formula).

At the end of the experiment, the bladders were irrigated for 30 minutes via a catheter using solutions with various citric acid and magnesium concentrations (Suby-G[®] and Solution-R[®]). Additionally the solutions differed in acidity (Table 2). The catheter drainage capacity (measured as draining volume per 45 seconds by a starting water column of 15 cm) and the dissolution capacity (described as relative part of dissolved encrustation material determined by chemical analysis) of infection-induced stone material in comparison with non irrigated controls was determined.

The catheters were examined by scanning electron microscopy (SEM) [33]. The inner and outer surface of the catheter was documented for new catheters, after experimental use, and after use and following irrigation. Energy dispersive X-ray microanalysis was carried out for selected encrustation areas on the catheter surface (inside, outside, balloon, lumen sediment). The catheters were also documented by photography as new catheters, at the end of the experiment, and after use and irrigation.

Results

Microbiology

Microbiological results are described as changes in bacterial concentration, ammonium concentration and

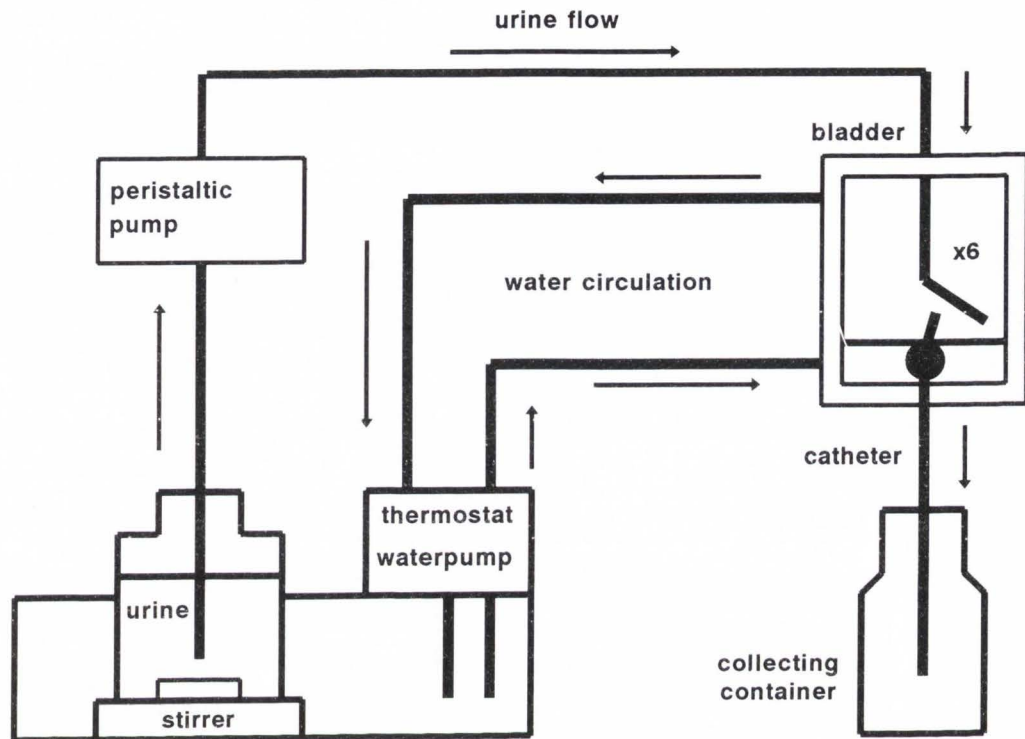


Figure 1. Schematic presentation of the 'bladder model' with urine flow direction, water circulation, thermostat, stirrer, urine container, reaction room ('bladder') and catheter.

pH-values at the start (time = 0 hour) and the end (time = 30 hours) of each experiment. The bacterial concentration of *Proteus mirabilis*, inoculated in the bladder, increased significantly from 10^7 to 10^9 c.f.u./ml during the experiment (with a minimum concentration of 10^4 c.f.u./ml at four hours).

Concerning the urease activity of the *Proteus mirabilis* strain used, ammonium concentration also increased significantly from 18.5 mmol/l to 228.0 mmol/l. The variation in fresh synthetic urine before inoculation with bacteria (CV = 1.9%) is smaller than after the 30 hour period of experiment (CV = 7.8%). At the same time, urinary pH-values increased significantly from 6.3 to 9.0 and created, in connection with the high ammonium concentration, conditions for infection-induced stone formation (Figure 2).

Urine analysis

Urine analysis for magnesium, phosphate, calcium and oxalic acid concentration showed significant changes during the 30 hour experiment ($p < 0.05$). The magnesium concentration decreased from 3.2 to 0.7 mmol/l (with a variation coefficient after the experiment of 13.9%). Urinary phosphate concentration decreased from 23.6 to 15.1 mmol/l (the variation coefficient at the end of the experiment was 10.7%). Calcium concentration decreased from 4.4 to 2.8 mmol/l and oxalic acid from 0.15 to 0.13 mmol/l. Calcium concentration showed most variation at the end of the experiment (CV = 19.7%). Urinary oxalic acid concentration had less variation with a variation coefficient of 10.2% (Table 3).

Table 3: Changes in urinary composition (Mg, PO₄, Ca, oxalic acid) during 30 hours of experiment.

Content	mean (mmol/l)		CV (%)	
	start	end	start	end
Magnesium	3.2	0.7	1.7	13.9
Phosphate	23.6	15.1	2.5	10.7
Calcium	4.4	2.8	1.3	19.7
Oxalic acid	0.15	0.13	5.9	10.2

In summary, 78.1% of urinary magnesium supplied to the system was kept in the bladder, with a variation coefficient of 10.8%. Phosphate and calcium remained in the bladder for 35.9% and 36.9%, respectively (the variation coefficient was 17.2% and 15.3%, respectively). Only 11.1% of oxalic acid was kept in the bladder, with wide variation (38.6%) (Figure 3).

The relative supersaturation was calculated for struvite and apatite by the EQUIL computer program. The relative supersaturation (as a quotient of a_s / a_0 with a_s as the calculated urinary solution activity and a_0 as solution activity by state of saturation) for struvite and apatite increased significantly for struvite, from 1.3 to 93.9 and for apatite from 10^{-31} to 10^{-23} (Figure 4).

Encrustation analysis

Infrared spectroscopy analysis showed the presence of struvite, carbonate apatite, amorphous calcium phosphate, and calcium oxalate. Both fractions of calcium phosphate encrustations were joined to a single fraction of calcium phosphate, to reach a comparable

Encrustations on catheters

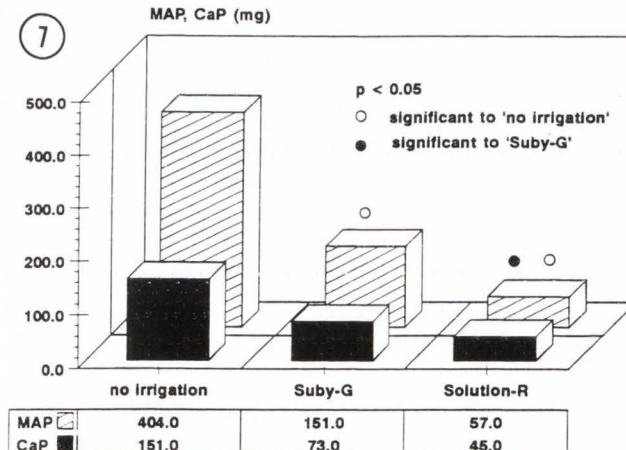
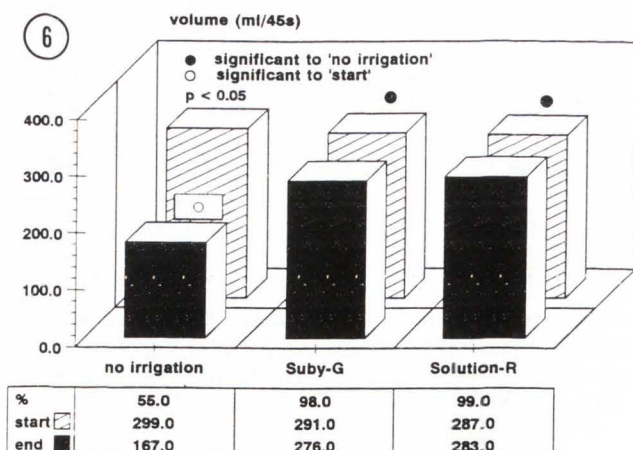
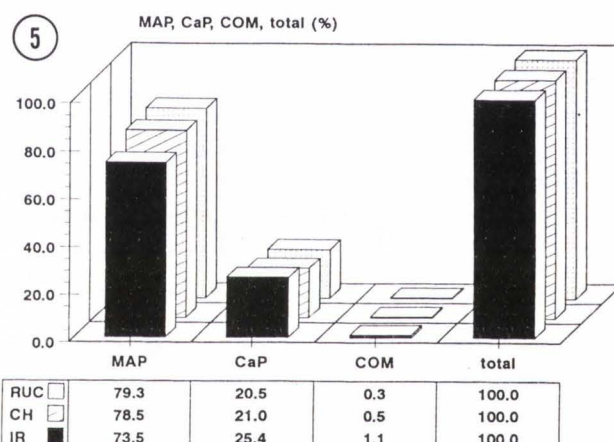
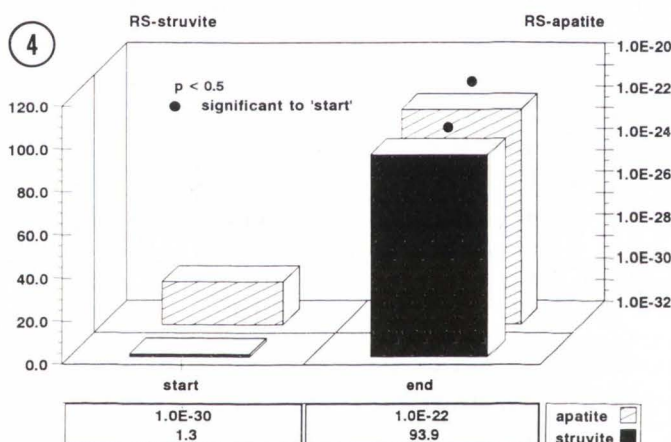
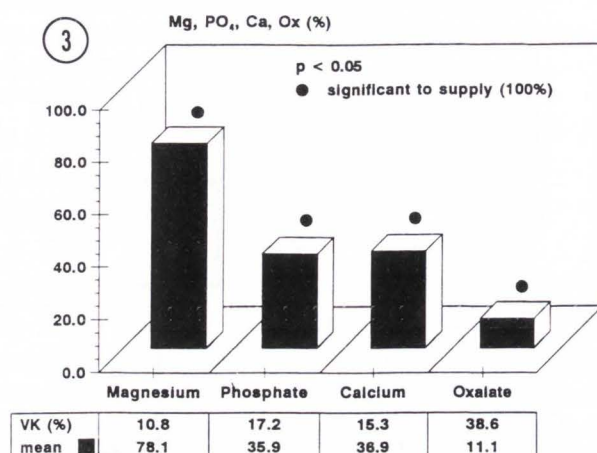
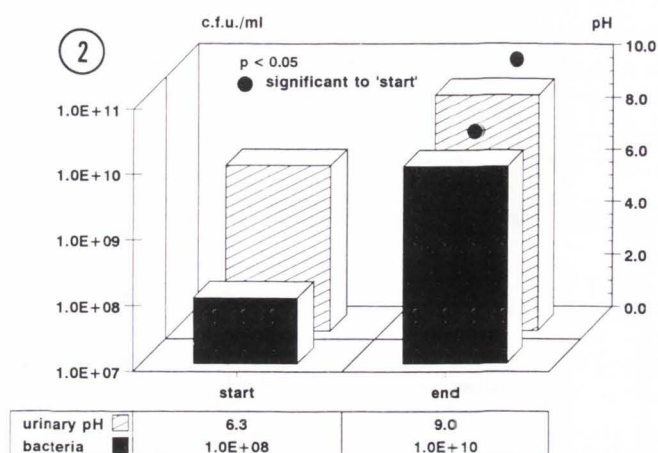


Figure 2. Changes in bacterial concentration (c.f.u./ml) and pH-value from start (time = 0 hour) to the end (time = 30 hours) of the experiment.

Figure 3. Percentages of magnesium, phosphate, calcium and oxalic acid remaining in the bladder as encrustation material during experiment in relation to the bladder supply (100 %).

Figure 4. Changes in the relative supersaturation for struvite and apatite during experiment, calculated by the EQUIL-computer program [10, 11].

Figure 5. Incrustations [magnesium ammonium phosphate hexahydrate (MAP), calcium phosphate (CaP) and calcium oxalate monohydrate (COM)] in the bladder (IR-analysis, chemical analysis, calculation by urinary losses).

Figure 6. Changes of drainage capacity during experiment (no irrigation) and after irrigation with citric acid containing solutions (Suby-G[®] and Solution-R[®]).

Figure 7. Dissolved mineral deposits struvite (MAP) and calcium phosphate (CaP) by citric acid containing solutions (Suby-G[®] and Solution-R[®]) on catheters.

Figure 8. Photograph of a new catheter, made of silicone coated latex catheter (inner and outer surface), size 18 Charr, filled with 10 ml. Bar = 1 cm.

Figure 9. Photograph of a catheter after the experiment without irrigation by citric acid containing solutions.

Figure 10. Photograph of a catheter after the experiment with irrigation by citric acid containing solutions.

presentation to the chemical encrustation analysis. 73.5% of the encrustation appeared as struvite, with lowest variation (CV = 10.5%). Calcium phosphate formed 25.4% of the encrustation with medium (CV = 21.7%) and calcium oxalate 1.1% with wide variation (CV = 76.1%). The chemical encrustation analysis corresponded with the IR-analysis. 1657 mg struvite (78.5%) with low variation (CV = 4.8%) and 447 mg calcium phosphate, calculated as carbonate apatite (21%; CV = 8.3%) were the main encrustation components. Calcium oxalate, calculated as calcium oxalate monohydrate, resulted in 9.6 mg (0.5%) with wide variation (CV = 34.1%) and had no important influence on the total encrustation of 2113.6 mg (CV = 7.1%).

Encrustation calculated by remaining urine contents in the bladder showed similar results. The calculation of struvite resulted in 1533 mg (79.3% with CV = 11.8%), and for calcium phosphate in 396.0 mg (20.5% with CV = 30.5). Calcium oxalate only resulted in 6.0 mg (0.3% with CV = 42.2%), so that the total mineral deposition resulted in 1934 mg (CV = 15.4%) (Figure 5).

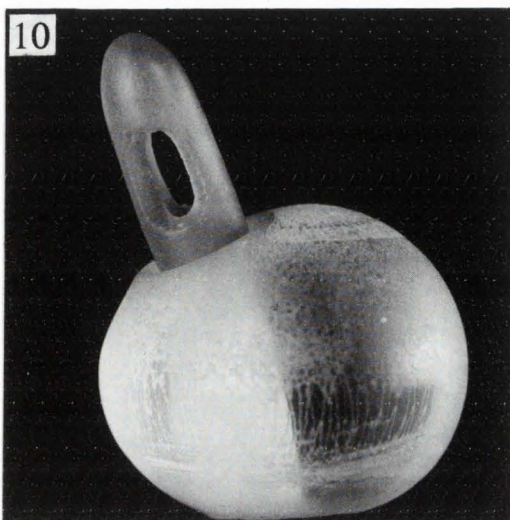
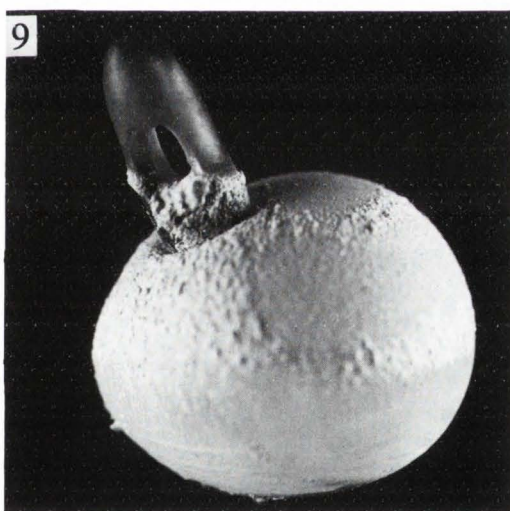
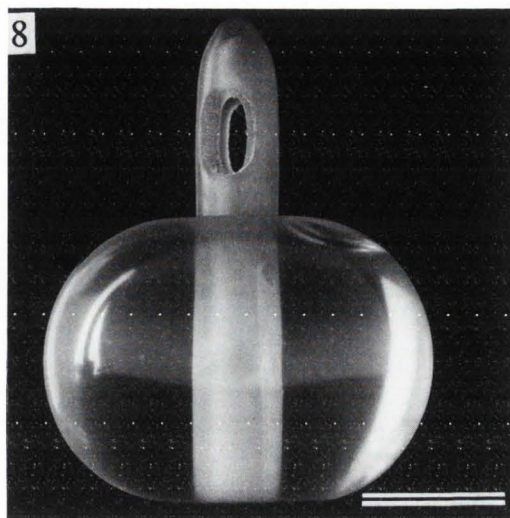
Irrigation solution

The catheter's drainage capacity significantly decreased during the experiment from 299 ml to 167 ml per 45 seconds. This was 55% of the drainage capacity of a new catheter. Irrigation treatment with the solutions tested, significantly increased the catheter's drainage capacity to 98% (Suby-G solution) and 99% (Solution-R solution) of a new catheter respectively. There was no statistical difference between new and irrigated catheters (Figure 6).

The solutions, tested in this investigation, showed a significant dissolution capacity for struvite and calcium phosphate in comparison to the non irrigated controls. Solution-R dissolved 85% of the struvite and 70% of the calcium phosphate and was significantly different from values of 70% and 55% respectively obtained with the Suby-G solution. Both solutions tested dissolved 30% of the calcium oxalate. This was not significantly different from the controls (no irrigation) (Figure 7).

Photographic documentation

The new catheters showed a macroscopically homogeneous surface with a transparent balloon (Figure 8). However, the catheter used without irrigation, was completely covered with encrustation material (Figure 9). The irrigated catheter carried less mineral deposition. There was a thin layer of encrustation material on the catheter surface (Figure 10).



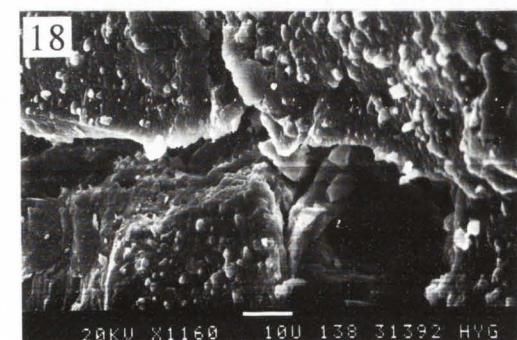
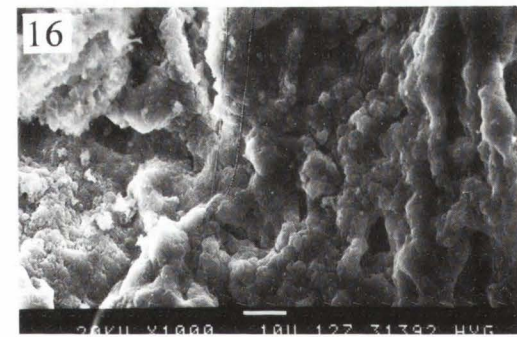
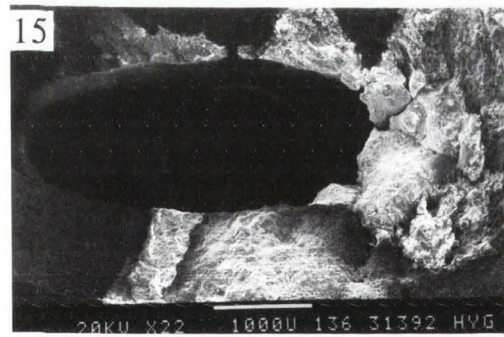
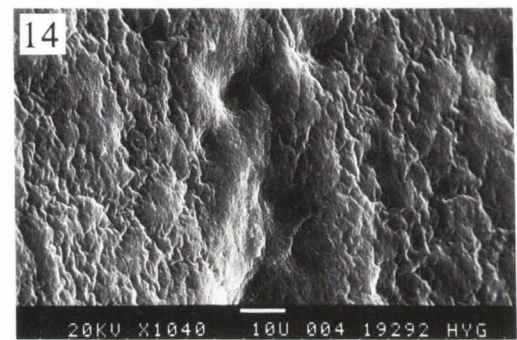
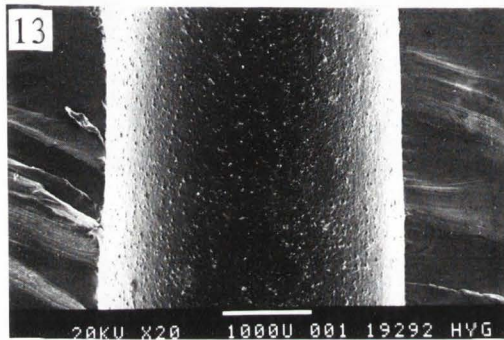
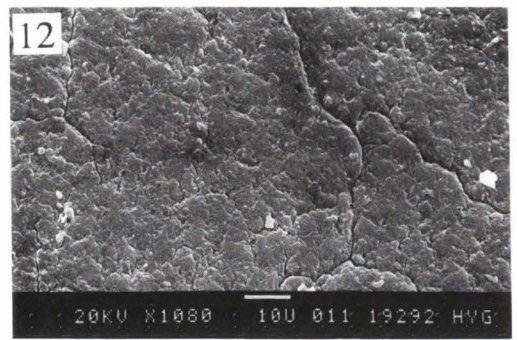
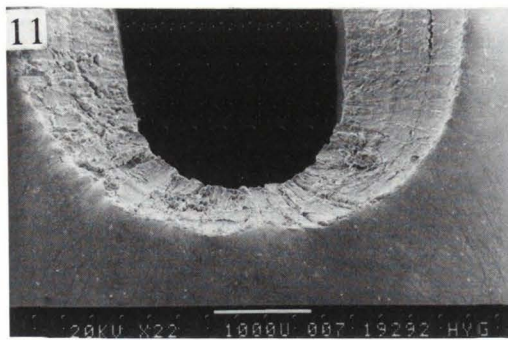


Figure 11. Outer surface of a new catheter (top) at low magnification (bar = 1000 μm).

Figure 12. Detail of the outer surface of the new catheter (eye; bar = 10 μm).

Figure 13. Inner surface of a new catheter (below the balloon; bar = 1000 μm).

Figure 14. Detail of the inner surface of the new catheter (bar = 10 μm).

Figure 15. Outer surface of the catheter after the experiment without irrigation at low magnification (bar = 1000 μm).

Figure 16. Detail of the outer surface of the used catheter (bar = 10 μm).

Figure 17. Inner surface of the catheter after the experiment without irrigation at low magnification (bar = 1000 μm).

Figure 18. Detail of the inner surface of the used catheter (bar = 10 μm).

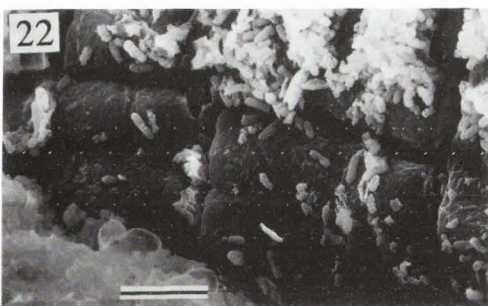
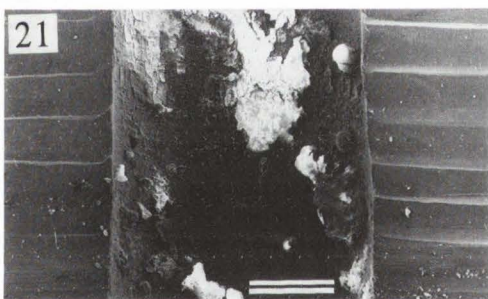
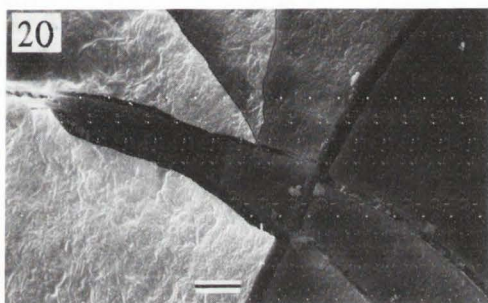
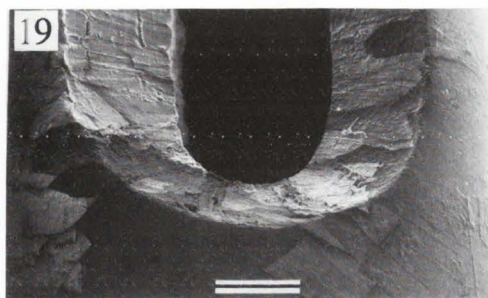


Figure 19. Outer surface of the catheter after the experiment with irrigation by citric acid containing solutions (bar = 1000 μm).

Figure 20. Detail of the outer surface of the used and irrigated catheter (bar = 10 μm).

Figure 21. Inner surface of the catheter after an experiment with irrigation by citric acid containing solutions (bar = 1000 μm).

Figure 22. Detail of the inner surface of the catheter after use and irrigation (bar = 10 μm).

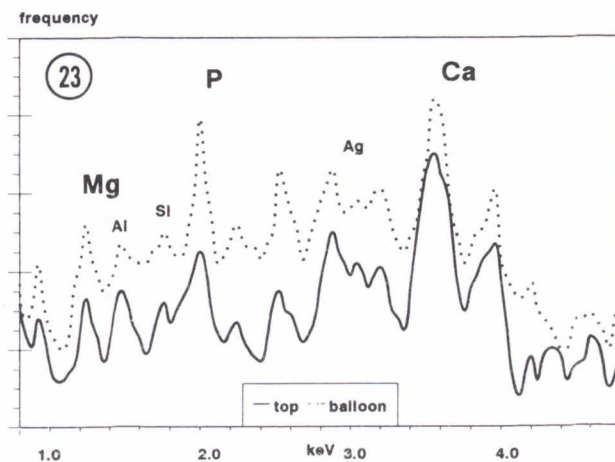


Figure 23. X-ray microanalysis of the surface of the catheter (top, balloon) with peaks for Mg, P and Ca from the encrustation material (Al from the sample carrier, Ag from the conductivity material, Si from the catheter-coating material).

SEM investigation

SEM investigation of the new catheters showed slight fissures on the outer catheter surface and a roughly punched hole for the catheter eye (Figures 11 and 12). The inside surface (Figure 13) however, was more uneven, but not cracked, as could be seen at higher magnification (Figure 14). Used, non-irrigated catheters showed large amounts of infection stone material and a bacteriological biofilm. The outside surface was completely covered with encrustation material (Figures 15 and 16).

The inside surface was also completely covered with encrustation material. The different thicknesses of the layers was a result of preparation. Because of preparation with a scalpel, loose encrustation material was removed from the inner surface of the catheters and separately examined as catheter sediment (Figures 17 and 18). Irrigated catheters carried less encrustation material. On the outside of the catheter, the encrustation appeared as a thin layer. The catheter was partly free of mineral deposits and its surface was visible. The outside surface of the catheter was cracked (Figures 19 and 20).

The inner surface of the catheter, after irrigation, showed single aggregates of encrustation material with intermediate encrustation-free areas and visible catheter surface. The inside surface was strongly cracked. Bacteria, partly in state of cell division, and bacterial biofilm were likely present (Figures 21 and 22).

X-ray microanalysis

Energy dispersive X-ray microanalysis showed obvious peaks for magnesium, phosphorus and calcium in all examined encrustations (Figure 23). The results support infrared and chemical encrustation analysis with struvite (magnesium and phosphorus) and calcium phosphate (calcium and phosphorus). There were also peaks for aluminum (due to the sample carrier material), silver (conductivity material) and silicone (due to the catheters coating material).

Discussion and Conclusions

The model established received reproducible results concerning formation of infection stone material. The standardized model led to conditions for the formation of infection stone material, especially struvite. Increase in bacterial concentration, pH-value and ammonium concentration provoked supersaturation of the struvite.

Encrustation analysis by infrared spectroscopy and chemical analysis showed similar results with more than 73% struvite in the bladder system. Calculation of encrustation by using losses of magnesium, calcium, phosphate and oxalic acid verified the model results. The chemical encrustation analysis had the lowest variation.

The SEM investigation showed struvite and calcium phosphate encrustation on the catheters and changes in structure of the surface of the catheter. Peaks for magnesium, phosphorus and calcium by X-ray microanalysis supported the encrustation analysis.

The tested citrate containing irrigation solutions nearly increased the drainage capacity of the catheter to their initial value. There was no significant difference between the two tested solutions concerning their effect on the drainage capacity of the catheter.

Both solutions tested had large dissolution capacities for infection stone material. The capacity increased with higher citric acid and magnesium concentration and with lower pH-value. The difference in dissolution capacity between the two solutions tested was significant. The dissolution capacity for calcium oxalate is low and equal for both solutions tested.

To reach reproducible qualitative and quantitative results concerning formation of infection-induced stone material, it is necessary to standardize the stone forming conditions. Standardization can be reached better *in vitro* (without physiological variation) than *in vivo*. On the other hand, transfer of *in vitro* results to *in vivo* conditions is difficult and often controversial. To overcome these difficulties, it is necessary to consider physiological conditions in the evolution of used models. If this is done, the results are comparable to the corresponding *in vivo* occurrences.

In the present study, the extensive simulation of physiological conditions permitted the formation of infection stone material similar to that *in vivo*, with comparatively modest variation in the same time. It could also be shown that formation of infection stone material depends on the surfaces involved (material and structure).

Acute infections with urease-producing bacteria can cause a complete seal of urinary catheters. However, acidic citrate containing solutions can be used to dissolve already existing encrustation material and, therefore, increase the catheters drainage capacity. But they cannot prevent the changes in the structure of catheter material (destruction of the smooth surface of the catheter). Solutions used to dissolve infection induced encrustation are a useful tool to prevent infection, to extend the catheter's life, and to minimize urothelium tissue injury by immediate removal after bladder irrigation. The treatment of patients with acidic solutions must be done with great care to prevent injury caused by the irrigation treatment.

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Discussion with Reviewers

J.A. Roberts: Formula $\{Ca_{10}(PO_4,CO_3)_5(OH,CO_3)_2\}$ is used in the chemical encrustation analysis for calculation of calcium phosphate, why?

Authors: As we knew from infrared analysis, we had two components of calcium phosphates (amorphous calcium phosphate and carbonate apatite), but we could not distinguish between them by chemical analysis. When handling all calcium phosphates as one group we used the formula which fits best with the proportions of calcium and phosphate in the encrustation material, and this is the formula mentioned in the text.

R.J.C. McLean: Are the solutions tested able to dissolve the bacterial biofilm which is formed as a layer on the encrustation material?

Authors: Your question cannot directly be answered by the results of our investigation. It is certain that the solutions dissolve the major part of the struvite and calcium phosphate (as shown by SEM and chemical analysis) and, also that a lot of bacterial biofilm disappeared (SEM). But we have not determined if the biofilm is really dissolved by the solutions or whether parts of it are removed from the surface of the catheter as a physical effect of the irrigation process.

Reviewer III: Bacterial studies are irrelevant to an *in vitro* system. Why did you make it in your system?

Authors: Bacterial studies are important to an *in vitro* system. To have a good control of the system, it is necessary to perform studies of the bacterial growth and activity. Furthermore, the main enzyme urease cannot be a complete substitute for the whole bacterium, because only with the use of bacteria can one produce the typical bacterial biofilm which highly influences bacterial adhesion, the conditions of stone formation, the stone formation itself and the removal of encrustation material.

A. Dumanski: Why was the experiment finished at 30 hours?

Authors: The encrustations started to form at 12 hours. After 30 hours the lumen of the catheters started to be closed by the encrustation material. Until this time, there was enough material for our analysis and the results were not distorted by unwanted effects, caused by closed catheters.

A. Dumanski: Why was the pH measured only at beginning and end?

Authors: When we started developing our model, we measured the pH every hour. The pH is constant for the first hours and increases from the fifth hour to the end of experiment from 6.3 to 9.0 first with an increasing gradient later with an decreasing one. When we knew the changes in pH during the experiment, we restricted our pH measurements to the beginning and the end to control the changes in urinary composition by bacterial activity.

G. Reid: Why were trypticase soy broth and dextrose nutrient solution used? Urine supports the growth of uropathogens, so it is unclear what the purpose was.

Authors: The synthetic urine includes only poor nutrition components for the uropathogens compared with human urine. However, the synthetic urine itself supports the growth of bacteria, but to get reproducible results in

encrustation, a constant and sufficient growth is necessary and this could only be guaranteed by addition of a nutrition solution.

H. Hedelin: How did you anticipate that the use of synthetic urine, and not human urine, and the addition of trypticase soy broth has influenced the results of the study?

Authors: The use of synthetic urine and the use of a nutrition solution was necessary in the study to reach our aim to get reproducible results in each case of the study. We tried to use human urine before and got the same qualitative results, but the quantitative results had a high variation, because the urine changed during the study. It is difficult to work with a large pool of human urine, because with a single flow through the bladder and a flow rate of 80 ml/h you need 2.5 liters per bladder. With six bladders in parallel, these are 15 liters of urine and that amount was needed 10 times in our study. The best way to get homogeneous results was the use of synthetic urine. It is reproducible: every time the urine has the same composition. Using the synthetic urine, the addition of a nutrition solution is necessary, because the synthetic urine itself is poor of nutrition.

G. Reid: The fact that an effect was found within 30 minutes of treatment might suggest a surface tension phenomenon which causes the encrustations to come off the materials used. What do you think about this?

Authors: The effect you describe is possible. After irrigation, plaques are visible in the solution, but their analysis shows none or only little struvite and/or calcium phosphate. The main part of this plaques is organic material. When, after irrigation, the infection-induced stone material is not on the catheter and cannot be found in the used solution, it must really be dissolved. This fits with the finding that after irrigation, the solutions contain additional magnesium and calcium. On the other hand, the finding that the plaques that come off the catheter material consist of organic material, supports the suggestion, that it is the bacterial biofilm which is not soluble itself.

A. Dumanski: How are the citrate containing solutions used in patients? How do they affect uroepithelial tissue? Is it a common practice?

Authors: In patients, the bladder is also irrigated via a catheter for 30 minutes with 100 ml of the solutions. This procedure is repeated every 48 hours and before the removal of the catheter. In our clinic, the solutions are also used for renal irrigation to attend the stone passage after extracorporeal shock-wave lithotripsy (ESWL) with percutaneous procedure.

H. Hedelin: The catheters used were silicone coated latex catheters. Were you able to evaluate, with the SEM studies, if the silicone coating was still present on the catheters at the end of the experiment?

Authors: The catheters used had a thin silicone layer on their outer and inner surfaces. After the experiment, this layer was removed in particular. We were able to evaluate this layer by the SEM studies as a structure different from the catheter surface and X-ray microanalysis showed only a weak signal or no signal at all for Si. The removal of silicone is caused by the 30 hours of the experiment and not by the irrigation, because we could also find partly removed silicone in non-irrigated catheters.

A. Dumanski: How were encrustations prepared for X-ray microanalysis?

Authors: We prepared the catheters for SEM and X-ray microanalysis by drying them in an alcoholic series (10, 25, 50 and 100% alcohol) to avoid artifacts to the greatest possible extent.

G. Reid: What are the surface properties of the *in vitro* bladder like? When the materials are different from human bladder, how can you make a correlation to the human bladder, when there is a different hydrophobicity and zeta potential, as in uroepithelial cells?

Authors: The surfaces are made of glass (side) and silicone (bottom) and, as you mention, consequently, are different from uroepithelial cells of the human bladder. The encrustation on these surfaces cannot be used to make correlations to the human bladder, but it is interesting, that different materials (glass, silicon and catheter materials) are different concerning their tendency to encrustation. The catheter is a foreign material and surface in the bladder which also appears in patients and to know its the tendency to encrustation is a very important information. To test the different catheters with regard to their tendency of encrustation gives a useful clinical indication.

A. Dumanski: Why is magnesium in the irrigation solutions when it is a major component of struvite?

Authors: We did not compose the solutions, we were testing them. However, it is a fact that a higher concentration of magnesium in the solutions, combined with decreased pH, increases its dissolving effect. Magnesium in the solutions and pH-values of about 4.0 increase the buffering capacity of the solutions. In this way the magnesium is no potent stone forming element. On the contrary it improves the uroepithelial tolerance to the solution.

H. Hedelin: Can you, on the basis of your results, suggest if and how often citrate solutions should be used in patients with indwelling catheters? Can a routine regime be drawn up or should it be performed on an individual basis?

Authors: To prevent the formation of infection-induced stone material, irrigation of the bladder of patients with indwelling catheters every 48 hours for 30 minutes can be a possible routine regime. With this treatment, it is possible to dissolve existing encrustation material and on the other hand, it will give no unexpected risk for damage to the uroepithelial cells by the acidic solution. Furthermore, the solutions have a bacteriostatic effect and can decrease the risk of UTI. An additional irrigation is useful before catheter removal. For renal irrigation you have to use the solution on an individual basis.

G. Reid: The final statement indicates that the citric acid solutions could cause patient injury, how? Are they currently being used to treat patients? Are they safe? Are the levels used here equivalent to those entering the bladder of humans?

Authors: The solutions have pH-values of about 4.0. An incorrect and too extended treatment can, therefore, damage the uroepithelial cells. We used the solutions in our clinic with good results for bladder irrigation and to dissolve renal stones. When they are used at levels as in the text (100 ml for 30 minutes), with repeated treatment in two day terms, they will be safe for the patients.