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Factors modulating the pH at which calcium and magnesium phosphates precipitate from human urine

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Abstract The factors controlling the rate at which crystalline bacterial biofilms develop on indwelling bladder catheters are poorly understood. It is known that normally the pH of voided urine (pH_v) is lower than the pH at which calcium and magnesium phosphates come out of urine solution (pH_n). In patients who develop infections with urease producing bacteria, however, the pH_v rises above the pH_n and precipitation of the phosphates occurs in the urine and the biofilm. The aim of this study was to examine ways of manipulating the pH_n of urine so that more of its calcium and magnesium remain in solution under alkaline conditions. The experimental data show that pH_n can be elevated by decreasing the calcium, magnesium and phosphate concentrations. Increasing the fluid intake of a human subject so that the urinary calcium fell from 120 mg/l to 25 mg/l, for example, resulted in the pH_n increasing from 6.48 to 8.22. The addition of citrate to urine also produced a rise in the pH_n. The daily consumption of 500 ml of fresh orange juice increased urinary citrate concentrations from 0.35 to around 1.21 mg/ml and the pH_n rose from 7.24 to 8.2. The pH_n of urine is thus a highly variable parameter. It can be manipulated by controlling the urinary concentrations of magnesium, calcium, phosphate and citrate ions. We suggest that increasing fluid intake with citrate containing drinks would reduce the extent of encrustation on catheters in patients infected with urease producing bacteria.

Keywords Urine · Catheter · Encrustation · Nucleation pH · *Proteus mirabilis* · Precipitation

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Introduction

A common complication in the care of the many patients undergoing long-term bladder catheterization is encrustation and blockage of the catheter. The problem stems from infection of the catheterized urinary tract by urease producing bacteria, particularly *Proteus mirabilis* [1, 2]. These organisms adhere to, and colonise the catheter surfaces forming biofilm communities of cells embedded in an adhesive polysaccharide matrix. The bacterial enzyme urease generates ammonia from urea and elevates the pH of the urine and the biofilm [3]. Under these alkaline conditions, crystals of magnesium ammonium phosphate (struvite) and calcium phosphate (apatite) are formed and become trapped in the organic matrix of the biofilm. The continued development of this crystalline biofilm eventually blocks the catheter lumen [4]. The flow of urine through the catheter is obstructed causing either incontinence due to leakage of urine around the catheter or retention of urine in the bladder. In the latter case, painful distension of the bladder and reflux of infected urine to the kidneys can culminate in episodes of pyelonephritis, septicaemia and shock [5].

Kunin et al. [6] classified patients who consistently and repeatedly developed extensive encrustation on their catheters as ''blockers''. Patients who did not suffer from encrusted catheters even after prolonged periods, were termed ''non blockers''. Blockers were shown to be infected with *P. mirabilis* and *Providencia stuartii* significantly more often then non blockers and to have more alkaline urine. The mean pH of urine produced by blockers was reported as 8.0 compared to 6.4 for non

blockers. Hedelin et al. [7] also examined the relationship between urinary pH and the precipitation of crystalline material on catheters. Their study revealed that patients who deposited appreciably quantities of phosphate salts on their catheters all had mean urinary pHs above 6.8. Burr and Nuseibeh [8] found that patients with spinal cord injury experiencing catheter encrustation had mean urinary pHs of 7.1 compared to 6.4 for non blockers.

Choong et al. [9] determined the pH at which precipitation of calcium salts occurred in single samples of urine from patients undergoing long-term catheterization. This value was termed the nucleation pH (pH_n) and for patients who were classified as non blockers, the mean pH_n was recorded as 7.66. The mean pH of the urine voided by these patients (pH_v) being 6.26, clearly explaining why deposition of calcium phosphate did not occur on their catheters. In contrast, patients who were blockers and infected by urease producing bacteria, had mean pH_v values of 7.85 and mean pH_n values of 7.58. The authors suggested that if the pH_n of urine could be made more alkaline, then patients would be less vulnerable to catheter encrustation. The aim of the work reported in this paper was to investigate methods of manipulating the pH_n of urine so that more of its calcium and magnesium salts remain in solution, under alkaline conditions.

Materials and methods

Bacterial strains and growth conditions

P. mirabilis SD0037, *Escherichia coli* SD0049, *Pseudomonas aeruginosa* SD0073, *Enterococcus faecalis* SD0108 are clinical isolates from patients attending the catheter clinic at the Urology Department of Southmead Hospital, Bristol. Growth was obtained on tryptone soya agar (Oxoid) at 37°C for 24 h except for *P. mirabilis*, which was grown on CLED Agar (Oxoid). Liquid cultures were grown overnight in tryptone soya broth (Oxoid) at 37°C in a shaking incubator (100 rpm).

Determination of pH_n of urine

The pH of a 200 ml volume of freshly voided urine sample of a healthy 35-year-old male was taken and reduced to 5.0 with concentrated hydrochloric acid. Using a 10 M NaOH solution, the pH was raised in 0.2 increments up to pH 10. At each increment, the optical density was measured at 550 nm, using distilled water as the blank, and a 1 ml volume centrifuged at 3,500 rpm for 3 min to remove precipitated material. The supernatant was diluted to 10%, 5% and 2% in nitric acid (5%), and concentrations of calcium and magnesium measured using atomic absorption spectroscopy (AAS). Phase contrast microscopy showed no evidence of particulate material such as microcrystals in the supernatant. As described by Choong et al. [10] plotting pH versus $[Ca^{2+}]$ or $[Mg^{2+}]$ produces two straight line segments which intersect at pH_n. The equations describing the two straight lines were calculated by least squares regression analysis.

Preparation of diluted urine

Increased oral consumption of water

The pH_n was determined for a 200 ml volume of early morning urine (EMU). Immediately following passing of the urine, 250 ml of bottled mineral water was consumed, and the pH_n then determined for the next urine sample passed. This was repeated with 500 ml and 750 ml volumes of water resulting in four samples taken during the course of the day, with decreasing solute concentrations.

In vitro dilution of urine

The pH_n for undiluted EMU, and urine diluted 1:2 and 1:5 with distilled water, was determined.

Manipulation of calcium, magnesium, phosphate, citrate, and pyrophosphate content of urine

All substances were tested at concentrations representing the normal range found in urine. In all cases, the second void of the day (termed midday urine, MDU) was tested with the exception of citrate for which both MDU and EMU were tested. A freshly voided urine sample was divided into 50 ml volumes which were supplemented with calcium to give a range of 0–200 μg/ml by the addition of calcium chloride (Sigma, Poole, UK). The pH_n of each was then determined by AAS measurements of $[Ca^{2+}]$ and $[Mg^{2+}]$. This was repeated using magnesium chloride (Mg concentration range of 0–200 μg/ml), sodium phosphate (phosphate concentration range of 0–10 μg/ml), sodium citrate (citrate concentration range of 0–10 mg/ml) and sodium pyrophosphate (pyrophosphate concentration range of 0–100 μg/ml). To test whether the addition of sodium and chloride ions might influence the results, pH_n was determined after the addition of sodium chloride (Sigma).

$[Ca^{2+}]$ and $[Mg^{2+}]$ of the untreated urine was measured by AAS, phosphate by colorimetric reaction with sodium molybdenum [11], and citrate using the citrate assay kit (Boehringer Mannheim, Darmstadt, Germany).

For citrate evaluation, increased oral intake was also used to elevate urinary concentration. During the week prior to the experiment, daily food intake was standardized to ensure, as much as possible, consistency of urine constituents. A total of 500 ml of freshly squeezed orange juice was consumed each evening at approxi-

mately 8.00 pm. The following morning, urine was collected, and tested for citrate concentration, and pH_n using AAS measurements of Ca^{2+}.

Preparation of bacterial cells and urinary sediments for pH_n determination

To test whether bacterial cells promote precipitation of Ca^{2+}, overnight cultures of *E. coli*, *P. aeruginosa*, *E. faecalis* and *P. mirabilis* (grown in tryptone soya broth at 37°C) were added separately to 50 ml volumes of freshly voided urine to give final concentrations of 10^4, 10^5 or 10^6 organisms/ml. In addition, urine samples from five catheterised patients with urinary tract infections were obtained from Southmead Hospital, and centrifuged at 3,500 rpm for 3 min. The sediments were added separately to 50 ml volumes of freshly voided urine from a healthy 35 year old, to give optical densities of 0.5 at 550 nm. The pH_n of each resulting urine preparation was then determined using AAS as outlined above and compared to that of the untreated urine

Results

The determination of pH_n of early morning urine (EMU) using NaOH to raise the urinary pH, along with optical density, Ca^{2+} and Mg^{2+} measurements, is shown in Fig. 1. Initial Ca^{2+} and Mg^{2+} urinary concentrations were 91.32 and 93.86 μg/ml, respectively. As urinary pH increased, $[Ca^{2+}]$ and $[Mg^{2+}]$ decreased, but only marginally. However, at a specific pH, supersaturation of urine occurred with these ions, and any further rise in pH was accompanied by substantial decreases in Ca^{2+} and Mg^{2+}. This pH defines the pH_n and it is apparent that the value differs depending on whether Ca or Mg determinations are utilised ($pH_n^{Ca} = 6.79$, $pH_n^{Mg} = 6.93$). In addition, the decrease in the concentration of ions was associated with a corresponding increase in optical density as amorphous phosphate salts were formed. It is evident that the plot of ionic concentration versus pH exhibits a biphasic relationship with the intersect defining pH_n. Continued pH increase resulted in a rapid reduction in $[Ca^{2+}]$ and $[Mg^{2+}]$; at a pH of 10 only 10% of the initial calcium and 20% of the magnesium remained in solution.

The effect of fluid intake on pH_n is shown by Fig. 2. The EMU sample has a high Ca^{2+} concentration, and a relatively low pH_n. As the urine became progressively more dilute due to the increasing consumption of water, the calcium concentration decreased and pH_n^{Ca} increased accordingly (Fig. 2a). Similar observations were made for the measurement of pH_n^{Mg} (Fig. 2b). This indicates that the pH_n for both Ca and Mg is dependent on their urinary concentrations. Similarly, dilution of urine in vitro using distilled water also resulted in a

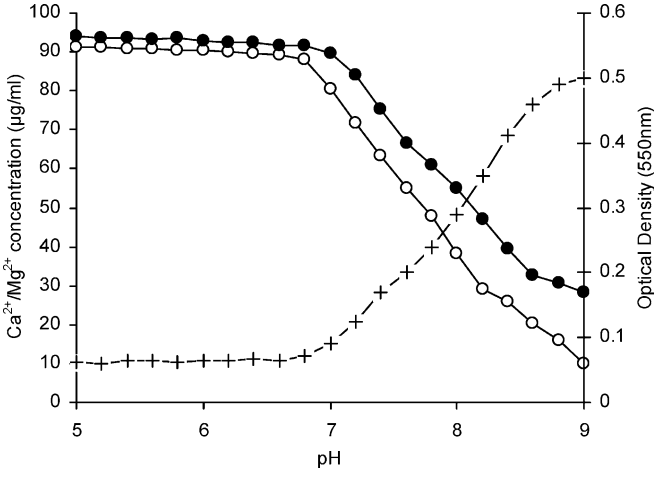

Fig. 1 Determination of pH_n^{Ca} and pH_n^{Mg} of urine using AAS and optical density (*pluses*) measurements. pH was sequentially raised by the addition of NaOH. The plots of pH versus $[Ca^{2+}]$ (*empty circles*) and $[Mg^{2+}]$ (*filled circles*) both describe a biphasic relationship, with the intersect defining the pH_n value. Least squares regression was used to create the straight lines of best fit

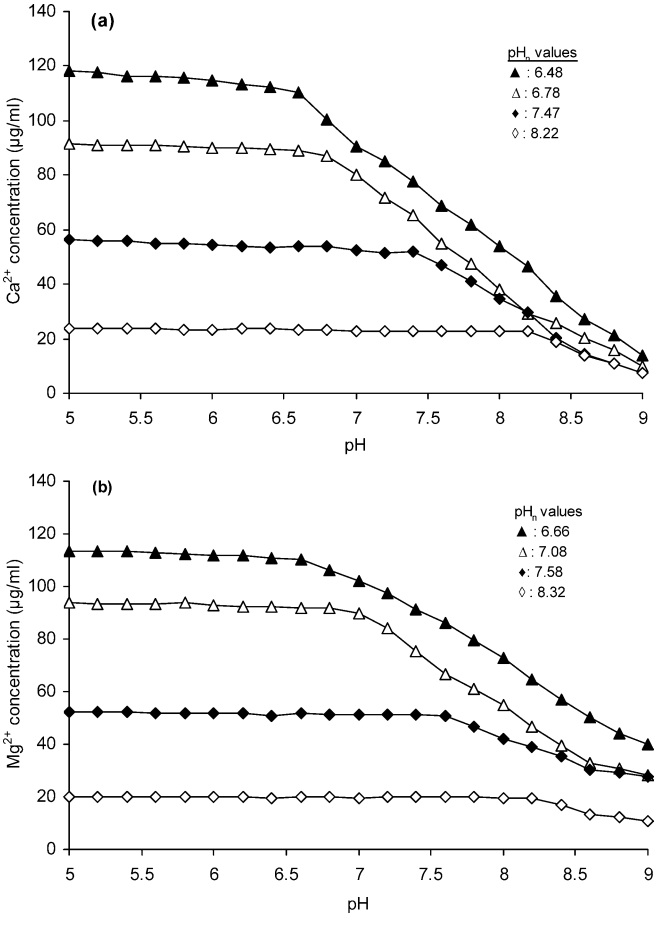

Fig. 2 Effect of increasing fluid intake on **a** pH_n^{Ca}, and **b** pH_n^{Mg}, as judged by AAS measurements of $[Ca^{2+}]$ and $[Mg^{2+}]$, respectively. EMU was tested (*filled triangle*), and then again after ingestion of 250 ml (*empty triangle*), 500 ml (*filled diamond*) and 750 ml (*empty diamond*) of bottled mineral water

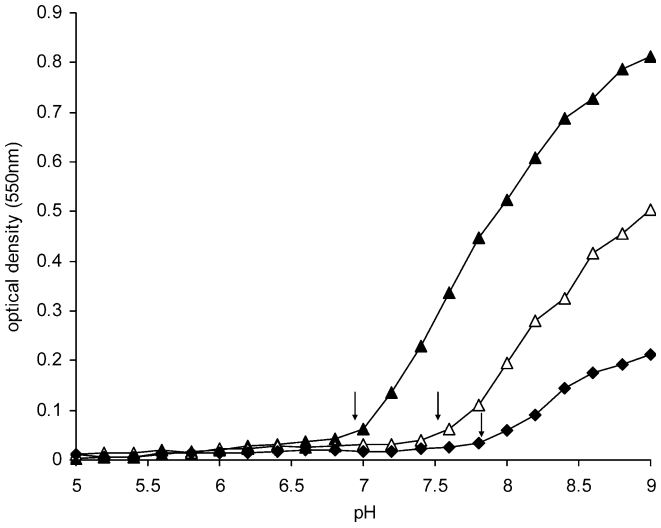

Fig. 3 Effect of the in vitro dilution of EMU using distilled water on pH_n, as judged by optical density measurements. The pH_n values, indicated by *arrows*, are 6.97, 7.52 and 7.82 for undiluted (*filled triangle*), 1:2 dilution (*empty triangle*), and 1:5 dilution (*filled diamond*), respectively. Plots of optical density versus pH describe a biphasic relationship as seen in Fig. 1 and 2. The pH_n values were determined using least squares regression

decrease in pH_n, as judged by optical density determination (Fig. 3).

The effects of the concentration of various urinary constituents on pH_n are shown in Fig. 4. The addition of Ca^{2+} resulted in a decrease in pH_n^{Ca} (increasing the urinary $[Ca^{2+}]$ by 100 μg/ml resulted in a pH_n of 6.52 compared to the value of 8.16 for untreated urine) suggesting that $[Ca^{2+}]$ is inversely correlated with pH_n^{Ca} (Fig. 4a). Similar results were observed for Mg^{2+} (addition of 100 μg/ml resulted in a decrease in pH_n^{Mg} from 7.73 to 6.82) (Fig. 4b) and phosphate (addition of 10 μg/ml resulted in a decrease in pH_n^{Ca} from 7.72 to 6.46) (Fig. 4c).

Citrate-induced inhibition of precipitation in urine is illustrated by Fig. 4d. In contrast to Ca^{2+}, Mg^{2+} and phosphate ions, the addition of citrate resulted in an increase in pH_n^{Ca}, presumably by chelating calcium ions. The pH_n varied from 6.5 (EMU; no citrate added) to almost 11 (MDU; 10 mg/ml citrate added) In contrast, the addition of a range of pyrophosphate concentrations to urine had little effect on the pH_n^{Ca} (Fig. 4e). The concentrations of Ca, Mg, P and citrate in the untreated samples tested were 37.7 μg/ml, 29.93 μg/ml, 20.88 μg/ml and 0.91 mg/ml, respectively. The addition of Na^+ and Cl^-, even at concentrations far in excess of those added to the test urine samples, did not result in a marked change in pH_n) (Fig. 4f). The effect which citrate concentration has on the pH_n of urine is also shown by Fig. 5. The oral consumption of orange juice resulted in increased urinary citrate concentration of from 0.35 mg/ml to 1.21 mg/ml. This was accompanied by a rise in pH_n from 7.24 to over 8.24.

The presence of bacterial cells in the urine did not affect the pH_n^{Ca} when compared with bacteria-free urine (Table 1); this was evident regardless of the species tested and culture density. Addition of the debris present in urinary sediments from infected catheterised patients also had little effect on the pH_n^{Ca} or pH_n^{Mg} values (Table 2).

Discussion

Up to 50% of patients undergoing long-term catheterisation experience recurrent blockage of the catheter, despite closed drainage systems and careful management [5]. While patients have been classified as blockers, or non blockers [12, 13], it is clear that catheter encrustation occurs at very different rates in different patients, with catheters blocking after periods ranging from 2 days to 6 weeks. The factors controlling the rate at which crystalline biofilms develop on catheters are poorly understood. It is clear that in non blockers the pH_v is lower than the pH_n for calcium. When a patient develops an infection with a urease producing bacterium such as *P. mirabilis*, however, the pH_v rises, exceeds the pH_n and salts precipitate in the urine and the catheter biofilm [9, 14]. Unfortunately, it is difficult to clear these organisms from the catheterised bladder. Urine acidification has been attempted using dietary vitamin C but proved ineffective due the difficulty of maintaining a low pH in the presence of urease producing microbes [2]. Other strategies to prevent or reduce the problems of catheter blockage have included replacement of the catheter, changing the size and type of the catheter, encouraging an increased fluid intake, drinking cranberry juice, bladder washouts with saline, acidic or antiseptic solutions and antibiotic treatment [9]. There is no evidence, however, from clinical studies that any of these methods are effective, and there remains a high incidence of catheter blockage, with no defined guidelines in place to advise nursing staff on how to deal with the problem. An alternative approach is to attempt to raise the pH_n so that it exceeds the pH_v, thereby maintaining calcium and magnesium in solution. In the present study, we have identified several key factors influencing the pH_n value, and then manipulated their urinary concentrations in an attempt to raise the pH_n.

Early work measuring the pH_n for calcium phosphate of patients urine, reported values of between 6.7 and 6.8 [12, 13]. Figures 1, 2, 3, 4, however, illustrate that the pH_n is variable, highly dependent on urinary [Ca], [Mg] and [phosphate], and directly related to fluid intake. The observation that the sequential dilution of urine throughout the day led to a corresponding increase in pH_n for both calcium and magnesium, suggests that pH_n may fluctuate significantly even from hour to hour. Langley and Fry [14] found that pH_n increased by 0.25, 0.56, 1.5 pH units upon dilution of urine by 90%, 50% and 10% respectively. Other studies have suggested that high concentrations of calcium and magnesium increase the likelihood of salt precipitation in urine and catheter biofilm [15, 16, 17].

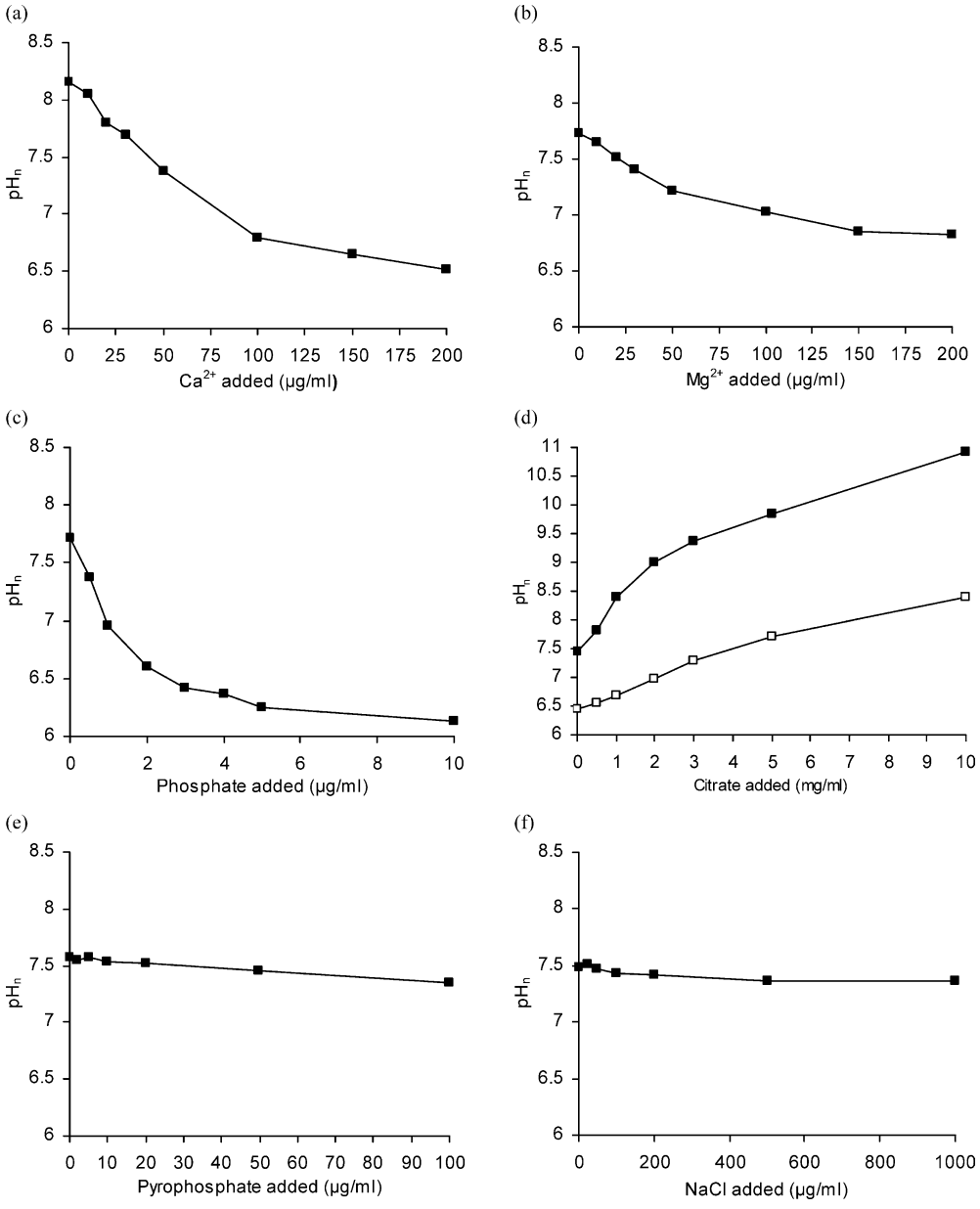

Fig. 4 Effect of **a** $[Ca^{2+}]$, **b** $[Mg^{2+}]$, **c** [phosphate], **d** [citrate], **e** [pyrophosphate], and **f** [NaCl], on pH_n^{Ca} or pH_n^{Mg} (Mg^{2+} only). For citrate (Fig. 5d) two plots are shown representing EMU (*empty squares*) and the next void of the day (MDU) (*filled squares*)

Urine is generally supersaturated with respect to Ca^{2+} and Mg^{2+} salts. These ions normally remain in solution because of the presence of complexing agents or substances that inhibit crystallization. Examples of these inhibitory substances include citrate, sulphate, pyrophosphate and the glycosaminoglycans chondroitin sulphate and heparin sulphate [18, 19, 20]. In an investigation on the ability of chondroitin sulphate, heparin sulphate and citrate to inhibit *P. mirabilis* induced crystal growth, only citrate was successful, and none of these agents had any influence on bacterial viability, pH or urease activity [21]. In the present study, a positive correlation was observed between urinary citrate concentration and pH_n in vitro (Fig. 4d). Also, an increased oral intake of citrate led to an increased urinary citrate content, although this concentration reached a steady level after 2–3 days, presumably due to the citrate metabolic regulation systems (Fig. 5). Pyrophosphate is another known inhibitor of crystallization and calcium oxalate formation [22, 23]. McLean et al. [18] demonstrated that pyrophosphate inhibited struvite growth, mainly by direct interference with chemical mechanisms involved in crystal nucleation and growth. However, here, pyrophosphate had little effect on calcium phosphate formation (Fig. 4e), although it is precipitation rather than crystallization that defines the pH_n value.

It has been proposed that crystallization is enhanced by the presence of bacterial surfaces such as bacterial cell walls and capsules acting as nucleation sites for crystal development and growth [24]. The bacterial cells themselves may encourage precipitation due to alkaline phosphatases releasing phosphate from organic molecules. However, the data reported in Table 1 suggest that the presence of bacterial cells does not encourage

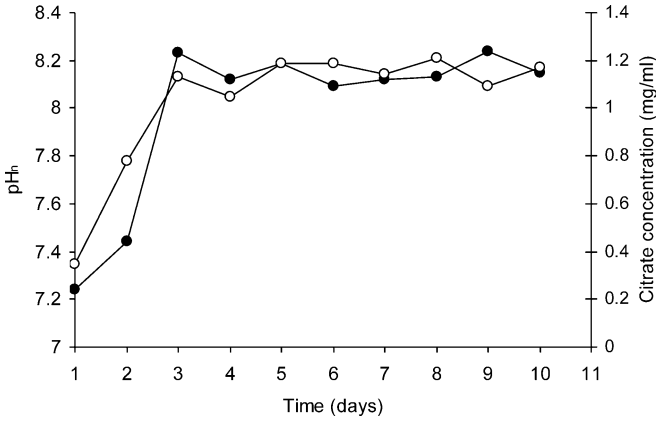

Fig. 5 Relationship between urinary citrate concentration (*empty circles*) and pH_n (*filled circles*) after increased daily oral consumption of citrate

Table 1 The effect of the presence and concentration of bacteria on the pH_n of urine

Origin of urinary sediment	pH_n^{Ca}	pH_n^{Mg}
No material added	7.28	7.35
Patient 1	7.3	7.35
Patient 2	7.27	7.3
Patient 3	7.38	7.32
Patient 4	7.26	7.38
Patient 5	7.32	7.36

Table 2 The effect of urinary sediment on pH n^{Ca} and pH n^{Mg}

Concentration of bacteria in urine samples (CFU/ml)	pH_n^{Ca} of urine			
	Escherichia coli	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Enterococcus faecalis*
0	7.53	7.4	7.58	7.29
10^4	7.48	7.49	7.58	7.37
10^5	7.55	7.43	7.56	7.33
10^6	7.52	7.39	7.61	7.3

precipitation of the salts. The non-dissolvable portion of catheter biofilms and urine consists of precipitated organic material, e.g. desquamated epithelial cells, leucocytes and bacteria [25]. The amount of this 'debris' varies between patients depending on age, UTI, underlying diseases, and can itself cause problems of catheter blockage, irrespective of urinary phosphate salt precipitation and deposition. The addition of this material from five catheterised patients to healthy urine did not affect pH_n (Table 2), suggesting that its presence does not promote precipitation in urine.

Burr and Nuseibeh [8] recommended that in order to control catheter encrustation in patients with spinal cord injuries, it is important to maintain a good fluid intake and avoid periods when urine becomes concentrated. Our data clearly supports this recommendation and further suggests that increasing urinary citrate levels would also reduce the extent of encrustation. While it is notoriously difficult to persuade elderly catheterised patients to maintain a good fluid intake, we suggest that efforts should be made to implement increased fluid intake with citric acid containing drinks for these patients.

In conclusion, the pH_n values for calcium and magnesium of an individual's urine are variable parameters. They depend on a variety of factors, most importantly urinary [Mg], [Ca], [phosphate], and [citrate]. The pH_n can be manipulated so that it exceeds the voided pH by altering the urinary concentrations of these factors. This will result in a reduced the precipitation of calcium and magnesium phosphates and, we suggest, will reduce the rate of development of the crystalline bacterial biofilms that so commonly encrust and block long-term catheters.

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