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Mineral Precipitation and Dissolution in the Kidney

Michael G Hill[1], Erich Königsberger and Peter M May
Chemical and Metallurgical Engineering and Chemistry
Murdoch University
6150 Murdoch,
Western Australia

Abstract

The formation of kidney stones is a significant human health problem. Calcium minerals are involved in a majority of these stones. Despite much research, the processes involved in stone formation remain poorly understood and hence, reliable procedures for preventing their formation have yet to be developed. However, recent advances point to some key steps in mineral formation and transformation involving calcium phosphates, which can help to illuminate these issues. A computer model has been developed to

[1] Author for correspondence

express the current status of literature data succinctly and to illustrate that computer modelling is a powerful tool for calculating mineral solubilities and for providing insight into the processes involved. Determining the nature of the initial solid phase of calcium phosphate formed is evidently important.

Keywords: Kidney Disease, Urolithiasis, Apatite, Brushite, Whewellite, Weddellite

Introduction

Urolithiasis denotes the pathological crystallisation of minerals that are deposited in the form of calculi or 'stones' in the urinary tract, especially in the kidney. In contrast to the biologically-controlled formation of bone and teeth, urolithiasis is a spontaneous process resembling the formation of minerals in low-temperature, aqueous geochemical environments. This review explores the thermodynamic and kinetic aspects of mineral-urine interactions, together with pathological preconditions of urolithiasis. Various calcium phosphate minerals are crucially involved in kidney stone pathology but many chemical and

mineralogical issues relating to them remain unclear. We summarize what is currently known and identify the most important areas for future work. Progress is unlikely unless current understanding can be made more quantitative.

Kidney stone formation is a worldwide problem (Linder and Little, 1986; Grases et al., 1999; Moe, 2006), and is very painful (Grases et al., 1998; Thomas and Hall, 2005). There is a high economic cost associated with the condition as a result of hospitalization and days taken off work (Linder and Little, 1986; Grases et al., 1998, 1999; Parks and Coe, 1996). Although surgical treatments have improved, there is a high, and increasing, incidence of the pathology (Romero et al., 2010; Tiselius, 2011b). Despite much research, the underlying causes are still not well understood; prevention has therefore proved difficult (Söhnel and Grases, 1995; Grases et al., 1998; Grases and Costa-Bauza, 2006; Evan et al, 2015; Tiselius, 2015).

Some risk factors are, however, well known. Incidence is age and gender dependent, being twice as common in males than in females (Hesse et al., 1986; Moe, 2006; Hughes, 2007; Romero et al., 2010; Tiselius, 2011b), with a peak age of presentation at 20 to 50 years (Robertson et al., 1981; Hesse et al., 1986; Hughes, 2007). Dietary factors are

significant, especially increasing risk are diets high in animal protein (Abdel-Halim, 2005; Tiselius, 2011b) and fat (Tiselius, 2011b). Insufficient fluid intake, resulting in a more concentrated urine significantly exacerbates the problem (Tiselius, 2011b). Obesity is another well known risk factor (Abdel-Halim, 2005; Hughes, 2007; Romero et al., 2010; Tiselius, 2011b; Rendina et al., 2013). The environment also has an effect: risk is increased for those living in hot climates and in periods of hotter weather (Soucie et al., 1994; Moe, 2006; Hughes, 2007; Romero et al., 2010). Genetic influences are known to be important and differences have been noted in rates of urolithiasis between different racial groups. Incidence and prevalence is highest in Caucasians, decreasing in Hispanics and Asians and lowest in Africans (Soucie et al., 1994; Hughes, 2007; Romero et al., 2010; Moran, 2014). In fact, kidney stones are very rare in most of Sub-Saharan Africa (Kumar and Muchmore, 1990; Rodgers, 2006).

Physiology

The kidneys perform the vital function of removing unwanted substances from the blood. To understand kidney stone formation it is necessary to consider first the processes of filtration and reabsorption. The balance between these two plays a key role in the potential

nucleation of stone forming minerals. Filtration starts with an unselective separation, where the fluid that is blood plasma passes through an ultrafiltration membrane into the tubules of the kidney. This is then followed by (a) a selective reabsorption process, in which metabolically useful substances are returned from the filtrate back into the blood, and (b) secretion, in which unwanted substances are transferred into the fluid in the tubule, and thus ultimately become excreted in the urine.

The basic functional unit of the kidney is called a nephron. A nephron is a tube, through which flows the fluid being processed by the kidney. Each nephron consists of a number of sections for adding or removing substances from the fluid in the tube to perform the overall extraction of urine from the blood. The most important sections for present purposes are the Bowman's capsule (BC), the proximal tubule (PT), the loop of Henle (LH), the distal tubule (DT), the collecting tubule (CT) and the collecting duct (CD). The loop of Henle is made up of a thin descending limb, a thin ascending limb and a thick ascending limb, as shown in Figure 1. Nephrons vary in length. The 'long' ones have a longer loop of Henle and there will be some differences between the composition of the fluid in long and short nephrons. The output from a number of collecting ducts flows through the duct of Bellini, which is located in a papilla. The

papillae protrude into a calyx (CX), which is a space where urine collects before exiting the kidney via the ureter (Bell et al., 1968; Guyton and Hall, 2000; Kerr, 1999; Atherton, 2006b).

Although most of the filtrate entering the Bowman's capsule is reabsorbed, the reabsorption occurs unevenly along the length of the nephron (Atherton, 2006a). Some segments reabsorb more water than solutes, and reabsorption of the solutes takes place to varying extents in different sections (Guyton and Hall, 2000). This results in marked changes in solution composition and concentration as the fluid flows along the nephron (Asplin et al, 1996). As a result of the depletion of water, the solutes become more concentrated and in certain cases can become increasingly supersaturated with respect to various minerals.

The final result of the process is a solution containing all the substances to be excreted emerging at the urine-forming end of the kidney tubules.

Both the composition and daily volume of urine are very variable, both inter-individual and intra-individual (Saude et al., 2007). The pH of urine also varies from around 4.8 to 7.2 (Kok, 1997). Concentrations are dependent on daily urine volume which can vary significantly. Figures for typical daily urine volume range from 0.99 to 2.3 litres (Diem and Lentner, 1970; Taylor and Curhan, 2007; Eisner et al., 2010; Taylor

et al., 2010). The values in Table 1 have been calculated by dividing the average value in mmol per 24 hours by the volume to obtain concentration values for normal subjects.

Mineralogy

Minerals often occur naturally within biological structures. Multicellular entities are frequently made up of soft tissue supported by hard structures. In the case of vertebrates, these hard structures are normally composed of minerals, and biological mechanisms are generally required in order to construct and maintain these structures. Pathological calcifications, as in the formation of kidney stones and calculi formed in other parts of the body, such as the gall bladder, pancreas and salivary glands, may or may not involve active biological processes.

The minerals of particular relevance to this review are apatite, brushite, octacalcium phosphate, whewellite and weddellite. Apatite comprises a group of minerals with the general formula $Ca_5(PO_4)_3(F,Cl,OH)$ (Tiselius, 2011b). The minerals hydroxyapatite (hereafter abbreviated as HAP) and flouroapatite are found ubiquitously in the body as part of the

building blocks of bones and teeth (Söhnel and Grases, 2011). As a result of the need to form these structures, blood plasma, and many other biofluids, are supersaturated with hydroxyapatite (Taunton et al, 2010; Söhnel and Grases, 2011; Holt et al, 2014). Calcium compounds predominate in the majority of kidney stones; 85% of all kidney stones contain calcium salts. Most (about 80%) have calcium oxalate as the major component (Grases et al., 1999; Tiselius, 2011b). Other stones formed are typically either calcium phosphate or mixed calcium oxalate/calcium phosphate (Coe et al., 2011).

As well as being absorbed from food, oxalate (like uric acid) is a metabolic end product (Williams, 1978; Knight et al., 2006). An important function of the kidneys is therefore to excrete oxalate from the body. Given the well known insolubility of many oxalate salts, this introduces a range of possible precipitates. The calcium oxalate compounds predominantly found in kidney stones are whewellite (calcium oxalate monohydrate), and weddellite (calcium oxalate dihydrate). Calcium oxalate has three different crystal forms – the monohydrate (COM), the dihydrate (COD), and the trihydrate (COT). The literature frequently describes the monohydrate as the most stable compound whereas the trihydrate is considered to be metastable and the dihydrate unstable (Tomazic and Nancollas, 1980; Grases et al., 1998;

Rodgers et al., 2011). This is probably due to the fact that COD cannot be precipitated from solutions that contain only calcium and oxalate ions (Tomazic and Nancollas, 1980). However, COD can be precipitated from artificial and real urine and consequently often appears in kidney stones (Werness et al., 1979; Tomazic and Nancollas, 1980). The solubilities of these three hydrates follow the order COM < COD < COT (Streit et al., 1998). As a result, solutions saturated with either COD or COT are supersaturated with respect to COM. Both COT and COD transform into COM (Tomazic and Nancollas, 1980).

Kidney Stone Formation

The passage of fluid through the kidney causes significant changes in concentration and hence also ionic strength (Bell et al., 1968; Guyton and Hall, 2000; Atherton, 2006a). These changes, which can potentially result in supersaturation, are illustrated in Figures 2, 3, 4 and 5 (Asplin et al, 1996; Hojgaard and Tiselius, 1999; Kok, 1997; Rodgers et al, 2011; Tiselius et al, 2009), showing plots of published values of calcium, oxalate, phosphate and pH in different nephron segments. Kok gives probable ranges, shown as min and max in the plots. In fact, most urine samples are always supersaturated with respect to calcium oxalate and the calcium phosphates (Asplin et al., 1996; Grases et al., 1999).

It is known that hydroxyapatite is supersaturated throughout the length of the nephron and that there is a risk of calcium phosphate precipitation both in the ascending limb of the loop of Henle and the distal tubule (Tiselius, 2011b). Calculations have shown that precipitation of hydroxyapatite can cause the other salts to become unsaturated (Rodgers et al., 2011). However, it is not known which phase of calcium phosphate is the first to precipitate (Tiselius, 1997a). Our suggestion is based on Ostwald's Rule of Stages which holds that the formation of the least stable phases precedes the thermodynamically stable phase (Söhnel and Grases, 2011; Sawada, 1997): this identifies the first substance to precipitate in the formation of hydroxyapatite as one of (a) amorphous calcium phosphate (ACP), having the formula $Ca_xH_y(PO_4)_z \cdot nH_2O$, (b) octacalcium phosphate (OCP), $Ca_8H_2(PO_4)_6 \cdot 5H_2O$, or (c) brushite (Bru), $CaHPO_4 \cdot 2H_2O$ (Luptak et al., 1994; Asplin et al., 1996; Tiselius, 1997a; Grases et al, 1997; Söhnel and Grases, 2011). Knowing this initially-formed phase would obviously be important in establishing how the process of kidney stone formation begins.

Urinary supersaturation with calcium oxalate monohydrate is apparently never sufficient to result in homogeneous nucleation; thus, heterogeneous nucleation must be taking place on some nucleating substrate (Söhnel and Grases, 1995; Grases et al., 2012).

Hydroxyapatite, brushite, and uric acid are all likely candidates as substrates for calcium oxalate monohydrate nucleation (Robertson et al., 1981; Söhnel and Grases, 1995; Tiselius, 1997a; Højgaard and Tiselius, 1999; Tiselius et al., 2009; Grases et al., 2012).

Most calcium oxalate stones contain a small proportion of calcium phosphate, often in the core of the stone, indicating that calcium phosphate is a common initial crystal phase (Tiselius, 2011b; Højgaard and Tiselius, 1999). Recent work has suggested that calcium oxalate stone formation is based on calcium phosphate precipitation higher up in the nephron, which highlights the importance of understanding the particular mechanism involved (Tiselius, 2011a; Coe et al., 2011; Tiselius, 2015). High levels of supersaturation of calcium phosphate and higher pH can be found in the ascending limb of the loop of Henle and the distal tubule, especially in the long nephrons, which may in particular result in the precipitation of calcium phosphate (Tiselius, 2011a; Rodgers et al., 2011). Precipitated calcium phosphate may then either continue to move along in the nephron tubule, or be internalized by the nephron cells, in what appears to be a defense mechanism, hence building up solid in the interstitial tissue (Tiselius, 2011a). This precipitated calcium phosphate in the interstitial tissue acts as a precursor of 'Randall's Plaque' (Tiselius, 2011a) , which is a result of

tissue damage that is most likely associated with oxidative stress (Khan and Canales, 2015; Grases et al., 2015; Grases et al., 2016). Following loss of the normal urothelial covering of the renal papilla, the calcification of the interstitial tissue at the end of the nephron becomes exposed to urine, resulting in the formation of Randall's Plaque (Evan, 2010). There is thus strong evidence linking the presence of Randall's Plaque to the formation of attached calcium oxalate papillary kidney stones (Coe et al., 2011; Evan et al, 2015) since almost all calcium oxalate stones show some signs of attachment (Coe et al., 2011). In most cases the point of attachment is the papilla where the protective glycosaminoglycan layer becomes damaged or defective (Söhnel and Grases, 1995). These glycosaminoglycan layers have strong anti-adherent properties (Coe et al., 2011) so most calcium oxalate stones seem to be formed on Randall's Plaque instead. Indeed, the conditions required for the formation of the most common type of stone are the presence of Randall's Plaque and damage to the protective glycosaminoglycan layer (Tiselius, 2011b, 2011a).

When calcium phosphate crystals are transported further along in the nephron tubule, the influence of pH change becomes important. If the pH is sufficiently low in the collecting duct, the calcium phosphate which has remained within the nephron tubule dissolves and brings

about sufficiently high levels of calcium and oxalate concentration for crystal nucleation to occur (Kok, 1997; Højgaard and Tiselius, 1999). In the case where all of the calcium phosphate crystals dissolve, the resultant stone will be pure calcium oxalate but, a mixed stone results where some of the calcium phosphate remains undissolved. Whether, and how, the initial calcium phosphate precipitation can be counteracted is not yet known but has become an active focus of research (Tiselius, 2011b).

Besides the Randall's Plaque mechinism, there are two hypotheses to explain the formation of the initial entity that may lead to the formation of a kidney stone (Kok and Khan, 1994). In one model, the stone starts to grow as a free particle within the fluid in the kidney, and in the other the particle is attached from the outset to the wall of a duct within the kidney. Finlayson and Reid (1978) developed a quantitative model to describe fluid flow through the kidney and concluded that it was not possible for a kidney stone to form from a free particle. Kok and Khan (1994) examined the issue by updating the Finlayson and Reid model with more accurate data on nephron dimensions, differences between long and short nephrons, taking into account varying levels of oxalate concentration and considering the effect of crystal agglomeration, which had been left out of the original model. This study concluded that it

could be possible for a particle to grow large enough to become trapped within the transit time of fluid through the nephron provided crystal size is increased by agglomeration. Robertson (2004) further enhanced the model of Kok and Khan by including the effects of drag on fluid and particles travelling close to the wall and gravity acting on particles in upward draining nephrons. The results in this case indicated that even without agglomeration the particle may still become large enough to become trapped within the lumen before reaching the end of the nephron. In the alternative 'fixed particle model', crystals become attached, usually due to renal cell injury, at the opening of the duct of Bellini, where they may subsequently grow into the so-called 'Randall's Plugs' that obstruct the lumen of the nephron and result in stones often projecting into a minor calyx (Evans, 2010). The formation of Randall's Plugs generally requires abnormally high supersaturation with respect to HAP and COM (Khan and Canales, 2015).

People who suffer from calcium phosphate stones have been found to have decreased calcium reabsorption, as well as decreased HCO_3^- reabsorption in the thick ascending limb of the loop of Henle resulting in a higher pH in the distal parts of the nephron (Coe et al., 2011). As

calcium phosphate precipitates only at high pH, this leads to calcium phosphate crystals being preferentially formed in the collecting ducts. It has been shown that these stones can be almost completely made up of calcium phosphate (Tiselius, 2011b).

Those who suffer from calcium oxalate stones have been found to have decreased calcium reabsorption in the proximal tubule of the nephron (Coe et al., 2011). This results in high calcium concentrations within the loop of Henle and hence increased entry of calcium into the medullary interstitium and likelihood of calcium crystal nucleation in the thin limb basement membranes. The formation of Randall's Plaque is thus accellerated.

The Issue of Supersaturation

Supersaturation with respect to the stone constituents is a requirement for stone formation (Robertson and Nordin, 1976; Finlayson, 1978; Grases et al., 1999; Tiselius, 2011a). The composition of stones formed have been found to correspond to the supersaturation levels in the urine of the patient (Parks et al., 1997). Thus, knowledge of the state of saturation of various minerals in the ultrafiltrate as it passes through the

302 nephron is evidently essential for an understanding of the genesis of
303 kidney stones.

304 Urine is always supersaturated with respect to calcium oxalate
305 (Robertson and Nordin, 1976; Luptak et al., 1994). In the case of the
306 calcium phosphates urine supersaturation is not as frequent (Robertson
307 and Nordin, 1976) and is dependent on higher pH levels (Tiselius,
308 2011b).

309 Generally speaking, a number of carbonates (particularly calcium
310 carbonates) in biofluids appear to be supersaturated *in vivo*. However,
311 these calcium carbonates have not been found in kidney stones, even
312 though they are known to form sometimes in other organs – for example,
313 they can occur in pancreatic, salivary and gall bladder stones, where
314 vaterite, the least stable of these minerals has been found (Königsberger
315 and Königsberger, 2006). One possible explanation for this difference is
316 the more acidic pH of urine but a complete understanding of these
317 observations awaits elucidation.

318 The calcium oxalate hydrates are sparingly soluble substances
319 (Königsberger and Königsberger, 2006). The results of experiments to
320 determine the solubility of sparingly soluble salts can be influenced by
321 numerous factors including the techniques used to approach equilibrium

between solid and solution and physical characteristics of the sample, affecting particle size for example (Gamsjäger and Königsberger, 2003). Accurate measurement of the solubility of these salts is therefore difficult: published values of their solubilities are accordingly rather variable (Hodgkinson, 1980; Königsberger and Tran-Ho, 1997, Hummel et al., 2005).

The solubility products of sparingly soluble electrolytes are frequently measured as conditional solubility constants, or *concentration products* (K_{sp}), at constant ionic strength I (Gamsjäger and Königsberger, 2003). These values are functions of I and show specific ion effects at higher I (Figure 6). In a recent review (Hummel et al., 2005), solubility products for calcium oxalates have been critically evaluated and extrapolated to $I = 0$ (infinite dilution) using the SIT approach for the calculation of activity coefficients (see section below). In addition to the increase of CaOx solubility products with ionic strength as an effect of changing activity coefficients, Figure 6 compares selected experimental data for NaCl and KCl background electrolytes with recent critical evaluations (Hummel et al, 2005). A list of some values of solubility products at $I = 0$ ($K_{sp}°$) published for the calcium oxalate hydrates is also shown in Table 2.

342 The nature of urine increases the achievable supersaturation of the salts
343 of interest well above the measured experimental value for solubility of
344 sparingly soluble salts. Many ions present in urine, such as Mg^{2+}, citrate
345 and HPO_4^{2-}, increase the solubility of CaOx by forming complexes with
346 either the Ca^{2+} or the $C_2O_4^{2-}$ ions (Hodgkinson, 1980; Gutzow et al.,
347 1993; Streit et al., 1998). Such complex formation generally results in an
348 additional increase in solubility.

349 The solubility of CaOx in a 0.20 mol/L sodium chloride solution is 1.94
350 $\times 10^{-4}$ mol/L, while in an artificial urine solution this is increased to 2.98
351 $\times 10^{-4}$ mol/L (Streit et al., 1998).

For increasing concentrations of Mg^{2+} of 2.5, 5.0 and 7.5 mmol/L, the respective ion product values for calcium oxalate are 1.0×10^{-7}, 1.35×10^{-7} and 2.02×10^{-7} (Elliot and Ribeiro, 1973). Thus, urine contains much higher concentrations of calcium and oxalate in solution than are present in a saturated solution of calcium oxalate in water. In addition to the dissolved salts, urine contains macromolecules. A number of proteins and similar substances are secreted into the tubule by the tubular cells (Söhnel and Grases, 1995; Tiselius, 1997a; Højgaard and Tiselius, 1999). Tamm-Horsfall Protein (THP) is the most abundant protein in human urine (Devuyst et al, 2005), with a concentration of the order of magnitude of 10^{-7} mol/L in urine (Glauser et al, 2000; Laube et al, 2001); it therefore cannot bind a significant amount of calcium. While the macromolecules have sometimes been shown to promote crystal nucleation, they are also known to inhibit crystal growth (Rodgers et al., 1993). This process is mainly *via* the action of binding to calcium-rich centres on the crystal surface (Tiselius, 2011b). Phytic acid, present at micromolar levels in urine, is another substance that has been shown to inhibit the growth of calcium oxalate crystals (Söhnel and Grases, 1995), presumably by mechanisms akin to those mentioned above for macromolecules.

As already mentioned, the risk of stone formation can be determined from the supersaturated state of the stone forming salts. Methods involve measurement of ratios of concentrations of certain substances (Tiselius, 1997b) and determining how much is required to initiate precipitation following the addition of the ions of interest to a sample of urine. For example, adding calcium chloride or ammonium oxalate induces CaOx precipitation (Luptak et al., 1994; Laube et al, 2000).

Quantitative Chemical Speciation Modelling

It is now widely accepted that the application of geochemical techniques to "predict, identify and quantify minerals in low temperature aqueous environments can be adapted" to the study of biofluids (Taunton et al, 2010). Thermodynamic calculations have been used routinely to investigate the state of saturation of substances in urine (Linder and Little, 1986; Asplin et al., 1996; Parks et al., 1997; Laube et al., 2002; Königsberger and Tran-Ho, 1997; Milosevic et al., 1998; Rodgers et al., 2006; Pak et al., 2009; Rodgers et al., 2011) providing a useful alternative to the induction of precipitation by substance addition. This technique uses measurements of substance concentrations to estimate free ion concentrations and supersaturation states so that risk can be evaluated.

The most widely used program to perform such calculations has been EQUIL. This program was developed by Finlayson in 1977 (Finlayson, 1977; Brown and Purich, 1992). EQUIL2 is an updated version of this program, which included translation from FORTRAN to BASIC, making it available on a larger number of computers (Werness et al, 1985). Enhancements led to a newer version, EQUIL93 (Brown et al, 1994), which increased the number of ions and complexes that could be represented and updated the thermodynamic database with data from the Martell and Smith critically evaluated compilation of equilibrium constants (Martell and Smith, 1974-1982), and other sources.

A chemical speciation program (confusingly, also called EQUIL) was developed by Ting-Po and Nancollas in 1972 (Ting-Po and Nancollas, 1972), but this program appears to be unrelated to that described above.

Another program frequently used in urolithiasis research is the Joint Expert Speciation System (JESS) software package (May and Murray, 1991a, 1991b).

In addition to EQUIL and JESS, other software has also occasionally been used. Prywer and Mielniczek-Brzoska (2016) used HySS (Alderighi et al, 1999) to model chemical speciation in the formation of struvite kidney stones resulting from bacterial infection.

412

413 Grases et al. (1997) first used JESS to model the supersaturation of
414 calcium and magnesium phosphates in artificial urine in 1997. In this
415 work, citrate and oxalate were considered in addition to the inorganic
416 salts. All possible complexes whose formation constants were available
417 in the JESS thermodynamic database were thus considered. In addition,
418 one of the then built-in activity coefficient models of JESS was used
419 (Davies equation). Considering the number of species (213), reactions
420 (265) and thermodynamic quantities (more than 4000, including
421 enthalpy, free energy and heat capacity values), this urine model was
422 possibly the largest at that time. After incorporating solubility constants
423 (log K_{s0}) determined in their laboratory (Streit et al, 1998), Königsberger
424 and Tran-Ho (Königsberger and Tran-Ho, 1997) employed this model to
425 calculate solubilities of the three calcium oxalate hydrates in NaCl(aq)
426 and urine-like liquors. Subsequently, the JESS urine model was
427 extended to include uric acid and cystine (Königsberger and
428 Königsberger, 2001), resulting in a considerable increase in the number
429 of species (280), reactions (380), and thermodynamic quantities (some
430 7200, mainly equilibrium constants but also standard potentials, Gibbs
431 energies, enthalpies, and heat capacities). The effect of complexing
432 species such as citrate and magnesium ions on calcium oxalate

433 solubilities helped to identify conditions for reducing its supersaturation
434 in urine (Königsberger and Tran-Ho, 1997; Königsberger and
435 Königsberger, 2001). Significant effects of urine composition on uric
436 acid (Königsberger and Wang, 1999) and cystine (Königsberger et al,
437 2000) solubilities were not predicted nor found experimentally.
438 Furthermore, the JESS modelling suggested regions of thermodynamic
439 and kinetic control of calcium oxalate crystallisation that correlated well
440 with a clinical test (Grases et al, 2000).

441 In order to calculate the degree of saturation of a dissolved substance,
442 values for the ion activity coefficients have to be determined. A number
443 of empirical models can be used for this. The Davies equation (1) is an
444 extension of Debye-Hückel theory without adjustable parameters, it has
445 no theoretical foundation, but often works fairly well for ionic strengths
446 up to 0.1 mol kg^{-1} (Grenthe et al, 1997).

447 At 25º C,

448 $$\log_{10} \gamma_i = -0.51\, Z_i^2 \left(\frac{\sqrt{I_m}}{1+\sqrt{I_m}} - 0.3 I_m \right) \quad (1)$$

449 where:

450 γ_i is the activity coefficient of ion *i*

451 Z_i is the charge of ion *i*

452 I_m is the ionic strength on molal scale.

453 Specific Ion Interaction Theory (SIT), Equation (2), is a semi-empirical
454 model based on Brønsted-Guggenheim-Scatchard models. It contains a
455 number of parameters that have some theoretical basis (Grenthe et al,
456 1997).

457 $$\log_{10}\gamma_i = -\frac{Z_i^2 A\sqrt{I_m}}{1+1.5\sqrt{I_m}} + \sum_k \epsilon(i,k) m_k \quad (2)$$

458 where:

459 *A* is the Debye-Hückel parameter for activity coefficient

460 ε(*i*,*k*) are interaction coefficients for oppositely charged aqueous ions *i*
461 and *k*;

462 m_k is the molality of ion *k*.

463 The current method used by JESS is the SIT-like equation, shown in
464 Equation (3).

465 $$\log_{10} K' = \log_{10} K^0 + \left(\frac{-\Delta Z^2 A\sqrt{I}}{1+1.5\sqrt{I}}\right) + BI \quad (3)$$

466 where:

467 K^0 is the equilibrium constant at infinite dilution

468 K' is the conditional equilibrium constant at finite ionic strength

469 A and ΔZ^2 are the Debye-Hückel parameter and a function of the ionic
470 charges respectively

471 B is a temperature dependent parameter

472 (May, 2000)

473 The JESS software package calculates log(SI) values:

474 $$\log(SI) = \log \frac{IAP}{K_{sp}}$$

475 where

476 IAP is the ion-activity product

477 K_{sp} is the solubility product

478 Using these methods, estimates of supersaturation of the calcium
479 phosphate and calcium oxalate compounds have been calculated for
480 final urine, as well as for the different nephron segments (Robertson and
481 Nordin, 1976; Luptak et al., 1994; Tiselius, 1997; Rodgers et al., 2011;

Robertson, 2015). It has been determined that for the calcium phosphates, supersaturation and therefore the risk of crystallization is higher in the proximal and distal tubules (Luptak et al., 1994; Asplin et al., 1996; Tiselius, 1997a; Rodgers et al., 2011; Robertson, 2015). For calcium oxalate, supersaturation levels are higher in the collecting duct (Luptak et al., 1994; Rodgers et al., 2011; Robertson, 2015). The variation in the values on which these calculations are based, as discussed above, indicates that the quantitative results from such calculations cannot be regarded as exact. In general, computational models should be used to gain insight into the working of a process, rather than in attempts to obtain individual numerical results that can be taken as the definitive answer to the problem (May, 2015).

Using published data about concentrations of the solutes in the different nephron segments (Rodgers et al., 2011), shown in Table 3, some calculations performed using the JESS software package give the values shown in Table 4.

While some earlier work concentrated on the behaviour of minerals under simulated lung fluid conditions, with a focus of assessing mineral durability and secondary mineral formation (Taunton et al, 2010) we prefer to concentrate instead on the implications of Ostwald's Rule of

Stages (Chung et al., 2009), which is known to work well for systems which reach equilibrium too rapidly to apply conventional reaction path analysis, which is in constrast to the long-term time-frame for minerals resident in the lungs.

The results in Table 4 indicate that brushite is the supersaturated substance with the lowest SI value under the conditions in the distal portion of the collecting duct and thus, brushite seems from Ostwald's Rule of Stages to be the substance most likely to precipitate. Brushite has indeed been found in some kidney stones (Grases and Costa-Bauza, 2006), particularly in overgrowths of a calculus that had 'plugged' the duct of Bellini (Evan et al., 2015). The core of that specimen contained hydroxyapatite, the most stable calcium phosphate phase, which may well have been formed by recrystallization of brushite. Another instance of stone plugging in the duct of Bellini contained COD (Grases et al., 2016), which is less stable than COM. Both of these stones were associated with renal tissue damage probably acting as heterogeneous nucleant. We conclude that the crystallyzation of metastable phases according to Ostwald's Rule of Stages can be applied to the growth of stones on 'Randall's Plugs', which are usually associated with excessive supersaturation with respect to the stable phases (Khan and Canales, 2015). The metastable phases brushite and COD were also found in

523 cavities of low urodynamic efficacy, in which heterogeneous nucleants
524 (organic matter and calcium phosphate crystals respectively) become
525 trapped and high supersaturation is maintaned (Grases and Costa-Bauza,
526 2006).

527 In contrast, the growth of papillary stones induced by Randall's Plaque
528 inevitably proceeds even at the low supersaturation prevailing in urine of
529 normal composition. Such stones contain the stable phases HAP and
530 COM (Grases et al., 2015; Grases et al., 2016). However, (metastable)
531 amorphous calcium phosphates were found as possible precursors of
532 Randall's Plaque (Evan, 2010), which indicates high supersaturation and
533 the applicability of Ostwald's Rule of Stages in interstitial tissue.

534 As a result of these findings, we have developed a model to calculate the
535 concentration changes along the path of the nephron. The calculations
536 are based on published values of concentration, reabsorption and
537 excretion of different substances within the sections of the nephron
538 (Luptak et al, 1994; Asplin et al, 1996; Kok, 1997; Tiselius, 1997;
539 Hojgaard and Tiselius, 1999; Rodgers et al, 2011; Rodgers et al, 2013).
540 Output from the model for normal kidney filtration is shown in Table 5.
541 The model allows different conditions to be investigated by changing
542 input values which respresent blood plasma concentrations of the

543 substances under consideration and changes in how much of a particular
544 substance is reabsorbed in a given nephron section. For example, it has
545 been discovered that calcium oxalate stone formers often have reduced
546 calcium reabsorption in the proximal tubule (Coe et al, 2011; Worcester
547 et al, 2013), and the model allows simulation of such scenarios.

548 Using calculated concentrations for the different nephron sections
549 log(SI) values for substances of interest can be determined using JESS.
550 It should be stressed again that JESS calculates the chemical speciation,
551 and hence log(SI), by considering all complex species whose formation
552 constants are contained in its database.

553 Figure 7 shows log(SI) values for brushite for three different senarios,
554 normal kidney filtration with a plasma calcium concentration of 1.5
555 mmol/L and oxalate concentration of 1.75 μmol/L, a high plasma
556 calcium concentration of 3.0 mmol/L, and reduced calcium reabsorption
557 in the proximal tubule together with the increased plasma calcium
558 concentration. The second two situations result in an increased SI for the
559 brushite all along the nephron. Log(SI) for brushite is above zero in the
560 loop of Henle and the collecting duct, indicating an increased risk of
561 precipitation in those locations.

562

Figure 8 shows the log(SI) values for the calcium oxalate monohydrate for the same three conditions described above. This shows an increased risk of precipitation towards the end of the nephron.

Figure 9 shows the log(SI) values for the calcium oxalate monohydrate for normal, a high plasma concentration of oxalate of 3.0 μmol/L, and the same high value of plasma oxalate together with the reduction in reabsorption of calcium in the proximal tubule.

Both these simulations show log(SI) COM increasing in the proximal tubule to reach a peak in the ascending loop of Henle before decreasing toward the distal tubule, and then increasing steadily in the collecting duct. The risk of crystal formation, where log(SI) > 0, is only seen with higher than normal calcium or oxalate plasma levels, and increased with a pathological reabsorption profile. This is in good agreement with the results of Robertson (2015).

JESS Version 8.3 used in this work leads to the same general conclusion as the previous work by Rodgers et al. (Rodgers et al., 2011), although there are small quantitive differences due to changes in the way weak ion associations are handled (May, 2015). The absolute values of the saturations calculated by different JESS versions change to a small extent over time, but in almost all cases their pattern through the

different compartments is the same and no large discrepancies have been found. It can thus be concluded that the changes are due to updates that have been made to the database. Further information about how JESS approaches the selection of equilibrium constants is given in The JESS Primer, available via the website http://jess.murdoch.edu.au (May, 2015).

However, this issue is complicated by a number of theoretical and practical factors. These include the fact that protein binding of calcium ions is still not well characterised (Taunton et al, 2010; Holt et al., 2014), calcium buffering and the observation that coating of seeds by proteins can cause inhibition of crystal growth. Another factor is that the saturation state of relevant minerals may alter under physiological conditions (Miller et al, 1958; Streit et al, 1998). Note also that in this regard chemical speciation calculations using ion association frameworks have well known limitiations (May, 2015). The *absolute* SI values calculated by JESS are therefore interesting but need to be interpreted with caution. However, it is clear that their *changes* across the nephron are significant and must be taken into account.

602

603 **Implications**

604

605 Kidney stone formation is a serious medical problem for which the
606 underlying mechanisms are poorly understood. In the human body,
607 hydroxyapatite must be kept supersaturated to allow the processes that
608 form bones and teeth to operate. As a result of this, a number of calcium
609 phosphate minerals tend to be supersaturated in various biofluids. A
610 delicate biological balance is therefore required between preventing the
611 formation of solid structures where and when they are harmful and
612 producing them as required.

613

614 Understanding kidney stone formation requires the investigation of
615 mineral formation in a complex environment, where the changes that are
616 taking place are often very difficult to observe directly. Interdisciplinary
617 research in particular can be of great value in medical science.
618 Combining techniques from biology, geochemistry, thermodynamics,
619 mathematics and computer science, comprehensive models can now be
620 developed to investigate and explain processes taking place in the
621 human body. Computer modelling techniques are a powerful tool that

622 can be utilized to overcome the problems with experimental *in vivo* and
623 *in vitro* investigations. Thermodynamic calculations have been shown to
624 be useful, especially in improving understanding of the processes
625 involved in kidney stone formation. Much insight can be gained into the
626 processes taking place and the interactions between them. As more and
627 better data are included in the databases that these computer models use,
628 the results obtained from the models can be expected to improve. Basic
629 mineralogical theory and experiments provide the pre-requisite building
630 blocks for these databases. The modelling is then able to combine theory
631 and experiment to simulate the complex interactions between the
632 components of the system being investigated. Similar issues arise in
633 geochemical complex aqueous environments, where metastable
634 equilibria and kinetic restrictions often prevail. Insights obtained by
635 geochemical modellers may therefore also be helpful in improving the
636 computational area of kidney stone research.
637
638 Determining the details of the initial solid phase formation in the loop of
639 Henle, or distal tubule, would probably be of greatest value as this
640 information would help to show how the risk of this initial particle
641 formation can be reduced. Thus, investigation of crystal seed formation
642 is likely to be a key area for future research. Better understanding of the

643 thermodynamics, kinetics and morphology of the minerals involved is
644 therefore needed to improve prospects in this medical arena.
645

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Figure 1: Schematic Depicting the Nephron Structure and Function
Figure 2: Calcium Concentrations in the Nephron
Figure 3: Oxalate Concentrations in the Nephron
Figure 4: Phosphate Concentrations in the Nephron
Figure 5: pH Variation in the Nephron
Figure 6: Ionic strength dependence of Ksp for COM at 37 °C

Figure 7: Log(SI) Brushite with Increased Calcium

Figure 8: Log(SI) COM with Increased Calcium

Figure 9: Log(SI) COM with Increased Oxalate

Table 1: Substance Concentrations in Urine. A:Rodgers et al. (2006), B:Kok (1997), C:Diem and Lentner (1970), D:Siener et al. (2004)

Substance	Selected Concentration (mmol/L)	Selected Reference	Range (mmol/L)	References
Na^+	151	A	45 to 582	B
K^+	32.0	A	20 to 260	B
Ca^{2+}	2.25	A	0.5 to 7.5	B
Mg^{2+}	3.35	A	0.5 to 12.5	B
PO_4^{3-}	19.9	A	5 to 75	B
Cl^-	104.9	A	118.2 to 236.5	C
oxalate	0.108	A	0.1 to 1	B
sulfate	12.2	D	14.8 to 34.5	C
citrate	2.0	A	0.1 to 7.5	B
urea	338.3	C	206.7 to 469.2	C

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1006 Table 2: Published Values of CaOx Solubility Products At 37 °C

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Hydrate	$-\log K_{sp}°$	Reference
COM	8.65	Finlayson et al. (1990)
COM	8.55	Daniele et al. (1985)
COM	8.65	Streit et al. (1998)
COM	8.65	Hodgkinson (1980)
COD	8.30	Finlayson et al. (1990)
COD	8.17	Streit et al. (1998)
COT	8.09	Finlayson et al. (1990)
COT	8.02	Streit et al. (1998)

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1012 Table 3: Nephron Concentration Data from Rodgers et al. (2011)

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Substance	Concentration (mmol/L)						
	GF	PT	LH	DTp	DTd	CDm	CDd
Na^+	135	135	278	79	93	94	109
K^+	3.8	3.0	13.8	0.90	58.0	53.0	63.7
Ca^{2+}	1.50	2.78	3.47	1.32	0.94	1.60	4.50
Mg^{2+}	0.54	0.19	0.24	0.12	0.40	1.45	3.85
PO_4^{3-}	0.80	0.80	1.00	1.00	3.34	12.1	32.3
oxalate	0.0015	0.01	0.013	0.013	0.04	0.12	0.32
citrate	0.07	0.09	0.11	0.11	0.37	1.21	3.21
SO_4^{2-}	1.4	3.1	3.9	3.9	13.0	7.8	20.8
Cl^-	139	139	293	101	145	146.6	170.0
pH	7.40	6.75	6.50-7.30	6.38-7.00	6.45-7.00	5.00-6.25	5.50-6.70
av pH	7.40	6.75	6.90	6.69	6.725	5.625	6.1

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1020 Table 4: log SI Values for the Stone Forming Salts

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		Salt			
	pH	CaOx	Bru	HAP	OCP
GF	7.40	-1.267	-0.592	9.043	1.754
PT	6.75	-0.236	-0.458	7.365	1.118
LH	6.50	-0.291	-0.643	5.972	0.230
LH	7.30	-0.294	-0.516	9.546	2.208
DTp	6.38	-0.247	-0.726	4.413	-0.788
DTp	7.30	-0.256	-0.425	8.973	1.944
DTd	6.45	-0.145	-0.547	4.716	-0.328
DTd	7.00	-0.176	-0.400	7.294	1.183
CDm	5.00	0.506	-0.921	-1.694	-4.096
CDm	6.25	0.438	0.035	5.970	1.172
CDd	5.50	1.084	0.184	4.212	0.550
CDd	6.70	0.853	0.681	9.961	4.182

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1029 Table 5: Nephron Concentration Data from the Model

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Substance	Concentration (mmol/L)						
	GF	PT	LH	TAL	DT	CD	CX
Na^+	145	146	324	121	129	22	124
K^+	4.2	1.9	8.0	1.0	1.5	5.5	30.6
Ca^{2+}	1.5	1.7	4.6	1.7	0.7	0.7	2.3
Mg^{2+}	0.4	0.7	2.3	0.6	0.9	0.3	1.7
PO_4^{3-}	1.5	1.0	1.5	1.5	2.2	3.6	20
oxalate	0.002	0.003	0.001	0.01	0.02	0.03	0.2
citrate	0.3	0.09	0.11	0.11	0.37	1.21	3.21
SO_4^{2-}	0.35	0.42	0.8	0.8	1.2	1.9	10.5
Cl^-	125	153	197	144	142	20	112
pH	7.4	6.75	7.0	7.0	6.45	6.25	6.0

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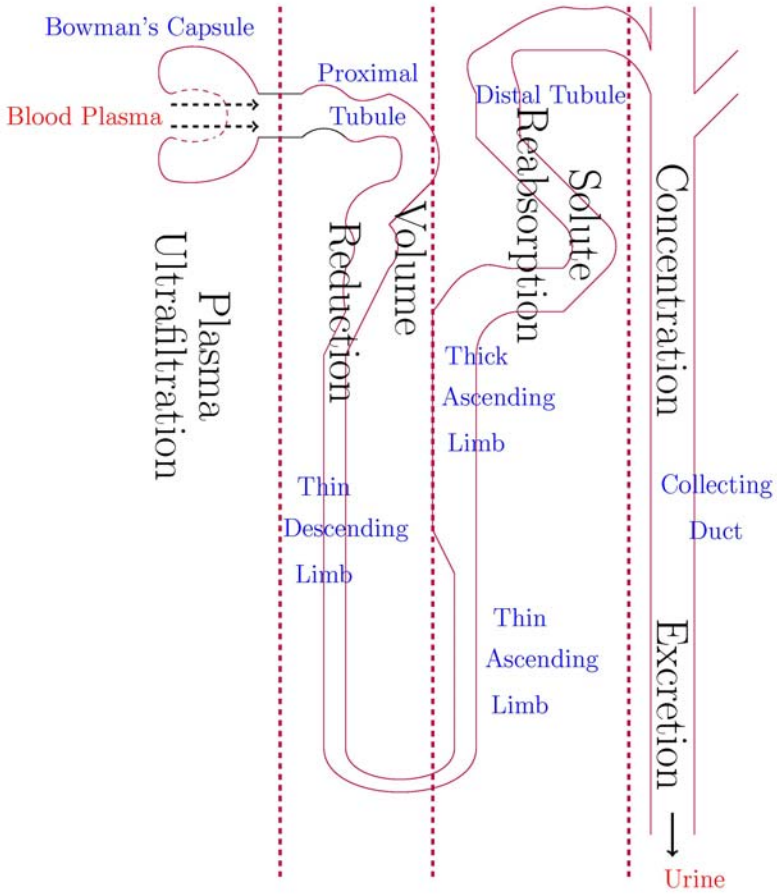

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1035 Figure 1: Schematic Depicting the Nephron Structure and Function

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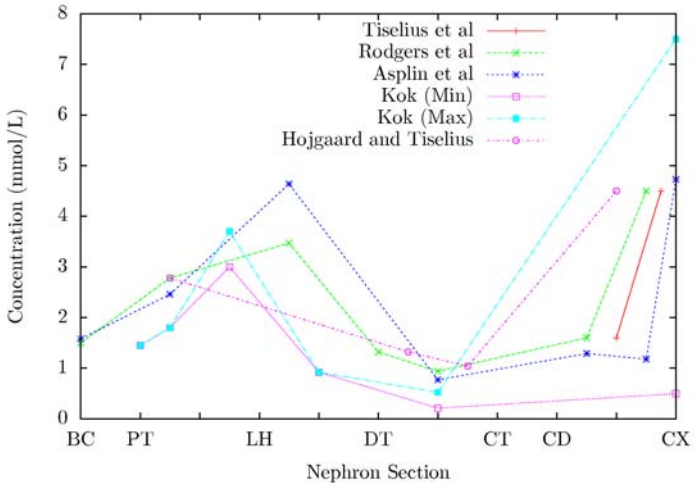

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1038 Figure 2: Calcium Concentrations in the Nephron

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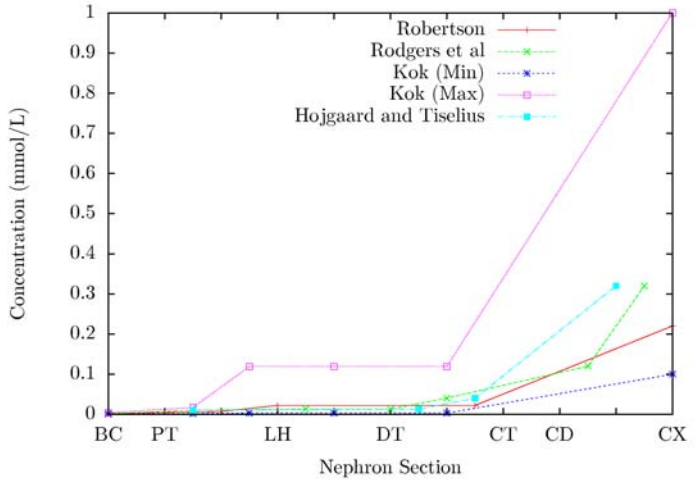

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1041 Figure 3: Oxalate Concentrations in the Nephron

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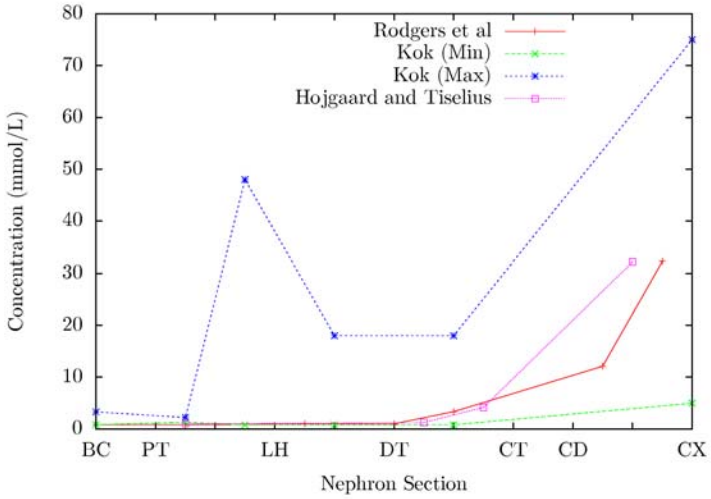

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1044 Figure 4: Phosphate Concentrations in the Nephron

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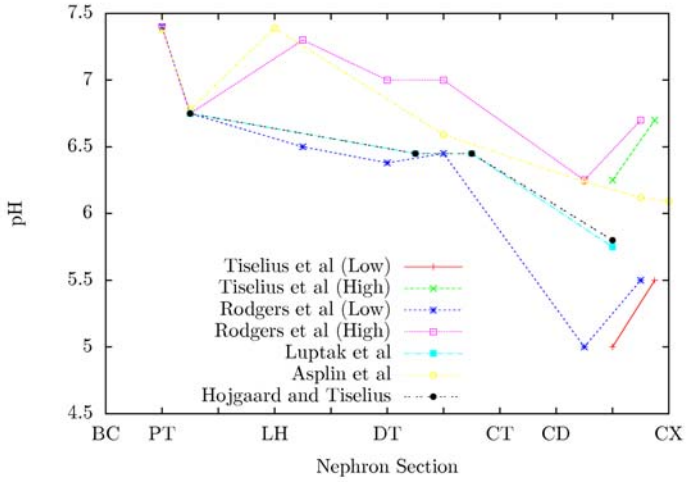

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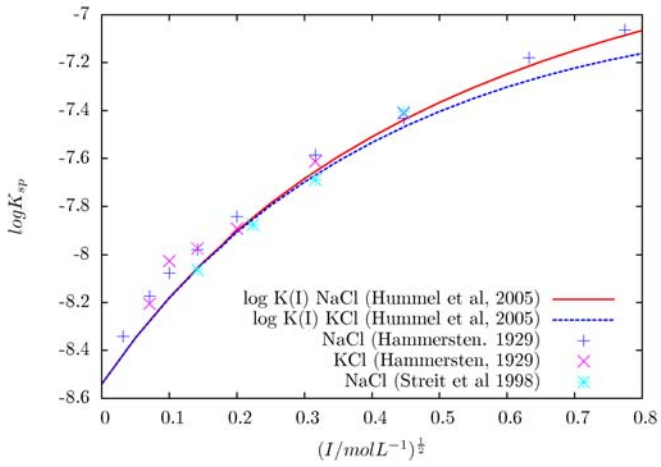
1047 Figure 5: pH Variation in the Nephron



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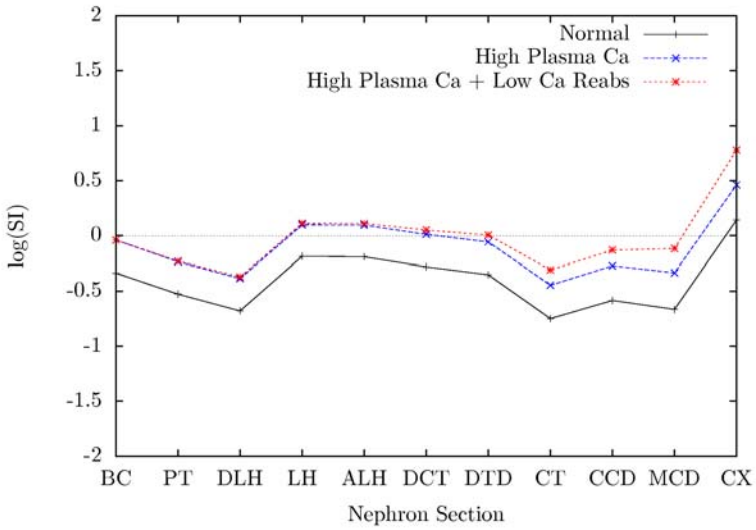
1049 Figure 6: Ionic strength dependence of Ksp for COM at 37 °C



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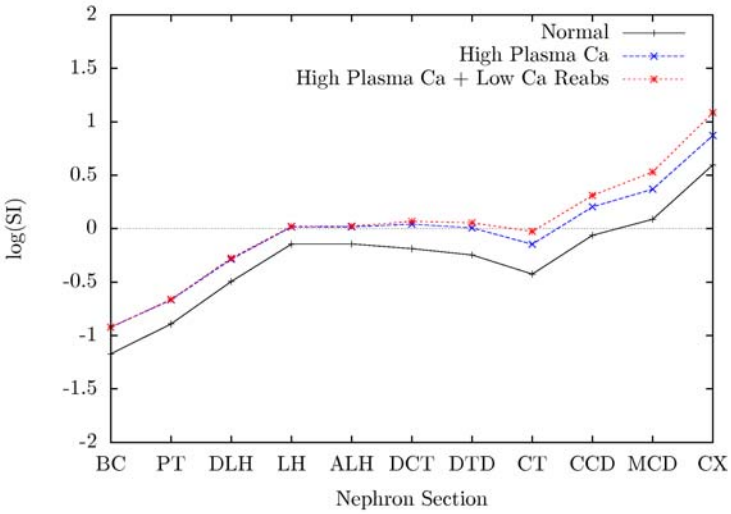
1051 Figure 7: Log(SI) Brushite with Increased Calcium



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1053 Figure 8: Log(SI) COM with Increased Calcium

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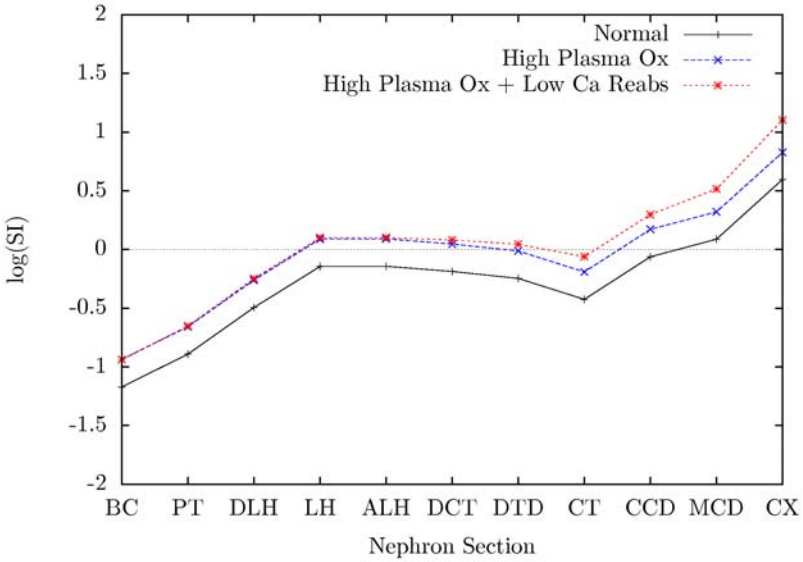

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1056 Figure 9: Log(SI) COM with Increased Oxalate