Chapter VI

SUMMARY

For the studies presented in this dissertation homogeneous and heterogeneous systems have been employed to study the rate and/or extent of mineralization and demineralization reactions. Calcium phosphate precipitation was taken as an index of mineral phase formation.

A simple, quick, homogeneous system of calcium phosphate precipitation has been described which can routinely be used in any clinical laboratory to assay the ability of given samples to influence mineralization and demineralization reactions. Based upon the ratio of the calcium and phosphate ions present in the precipitates, it was concluded that the precipitates formed resemble hydroxyapatite in nature.

Collagen isolated from sheep tendon, has been used to induce the uptake of Ca\(^{2+}\) and HPQ\(_4\)\(^{-2}\) from stable solutions under physiological conditions, to form matrix bound mineral phase which resembles hydroxyapatite in nature. As the incubation progressed more and more Ca\(^{2+}\) and HPQ\(_4\)\(^{-2}\) ions were removed from the reaction system to form matrix bound mineral phase. This process continued till the limiting velocity was attained at about 12 hr. The studies further revealed that the limiting velocity was obtained due to the attainment of Ca\(^{2+}\)x HPQ\(_4\)\(^{-2}\) product in the soluble phase that fails to support further ion uptake rather than the limiting
binding capacity of the catalytic matrix. The matrix once mineralized was not only found to act as a better catalyst to induce further mineral phase formation, but the matrix bound mineral phase ions were also found to participate in the iso-ionic exchange reactions with the corresponding ions present in the reaction system. Only 10-15 percent of the matrix bound ions were found to participate in isoionic exchange reactions.

Urine samples from 12 clinically normal male persons and 20 male kidney stone patients were used not only to study the effect of urine samples on mineralization and demineralization reactions but also to isolate and purify the potent biomolecules from the urine samples. Only those male kidney stone patients, who were suffering from no other abnormality and their stones were of non-recurrent and non-infectious nature were selected for the collection of urine samples. Their stones were detected by X-ray and confirmed by intravenous pyelography.

Studies presented in this dissertation demonstrate that on an average as compared to normal persons, kidney stone patients were found to excrete higher volumes of urine per day. On an average per day excretion of Ca$^{2+}$, creatinine, peptidal nitrogen and total carbohydrates were also found to be higher in kidney stone patients as compared to the normal persons. However, when the concentrations were expressed on mg/ml basis, kidney stone patients were found
to excrete significantly lower amounts of $\text{Ca}^{2+}$, $\text{HPO}_4^{2-}$, creatinine, peptidal nitrogen and total carbohydrates in their urine as compared to normal persons. Since for precipitation of ions as mineral phase it is the concentrations rather than total excretions which are important, the present studies could be interpreted to mean that factors other than the concentrations of the reactants in urine play an important role in the etiology of renal calculi formation.

Studies further revealed that in general, urine samples obtained from normal persons and kidney stone patients have the ability to not only inhibit the precipitation of ions as mineral phase (both in the absence and the presence of organic matrix) but also have the ability to stimulate the demineralization of the preformed phase. Stimulation of demineralization was observed when the nature of the preformed mineral phase was either calcium phosphate or powdered kidney stones. Experimental evidence has been presented to support the view that both the abilities to either inhibit mineral phase formation or stimulate demineralization of the preformed mineral phase reside in the same molecules and depending upon the concentration of reactants in the reaction system/bathing body fluids, one of the two properties can be expressed.

The studies further revealed that in general, as compared to normal persons, kidney stone patients were found to excrete significantly lower levels of potent
biomolecules in their urine. The inhibitory potencies of the urine from normal persons and kidney stone patients were also expressed in terms of inhibitory units (I.U). One inhibitory unit has been defined as the amount of urine which can inhibit the rate of collagen induced mineralization by 50 percent. On an average, normal persons and kidney stone patients were found to excrete $175\pm 84$ and $578\pm 50$ I.U/100ml of whole urine, respectively.

Urine samples obtained from 3 out of 20 kidney stone patients were found to stimulate rather than inhibit the extent of mineralization. No urine sample from normal persons was found to stimulate mineral phase formation.

The urinary inhibitory biomolecules were found to be soluble in water and methanol but insoluble in ether, chloroform and ethyl acetate. The studies further revealed that biomolecules excreted in the urine of normal persons and kidney stone patients which are responsible for the abilities of the urine samples to influence mineralization and demineralization reactions may be present in association with non-dialysable macromolecular moieties/moieties. The potent biomolecules could be released from this association by a pH dependent reversible phenomenon.

Conventional procedures (dialysis, ion-exchange, molecular sieve and FPLC) were employed to isolate and purify the potent biomolecules from the urine of normal persons and kidney stone patients. Experimental procedures employed during the present studies for purification of potent
biomolecules were such that only those biomolecules having molecular weights approximately between 2,000 to 15,000 were investigated. Following conclusions could be drawn from these studies:

1. Normal male human beings excrete at least five potent biomolecules in their urine. Two of these being relatively acidic in nature (molecular weights 2700 and 4000) and the other three being relatively basic in nature (molecular weights 3700, 6800 and 3250).

2. Male kidney stone patients excrete only three potent biomolecules in their urine. Out of these three, one (molecular weight 6600) being relatively acidic in nature and the other two (molecular weights 12,500 and 9200) being relatively basic in nature.

3. The relatively basic potent biomolecules isolated from the urine of normal persons and kidney stone patients were found to be polypeptide in nature.

4. The relatively acidic potent biomolecules isolated from the urine of normal persons and kidney stone patients were either present as free polypeptides or at least one of these polypeptides (both in normal persons and kidney stone patients) might be present bound to a moiety which was not only stable to acid treatment (6N HCl, 110°, 48 hr) but by itself could also inhibit mineral phase formation.
5. These potent biomolecules were found not only to inhibit the mineralization in homogeneous and heterogeneous systems but also to stimulate the demineralization of both calcium phosphate precipitates and kidney stone powders.

6. Identical elution profiles of the ability of urinary biomolecules to either inhibit mineralization or stimulate demineralization, observed at different stages of purification, indicate that these two properties may in fact reside in the same biomolecules and depending upon the experimental conditions, either one of the two properties can be expressed.

7. Potent biomolecules were also found to inhibit exchange of ions between the matrix bound mineral phase and the corresponding ions present in the reaction system.

8. As compared to normal persons, kidney stone patients were found to excrete much lower levels of the inhibitors in their urine and these differences between the normal persons and the kidney stone patients became more and more pronounced with purification.

9. When activities of the FPLC purified potent biomolecules were compared between the normal persons and the kidney stone patients, on equivalent protein basis, biomolecules isolated from normal persons were
found to be much more potent than their counterparts isolated from the kidney stone patients. Potent biomolecules III and IV isolated from urine of normal person and kidney stone patient were found to significantly differ in their amino acid compositions, thus suggesting that the difference observed in their activities may indeed be due to the differences in the primary structure of these polypeptides.

10. Phosphate was found to be associated only with the potent biomolecule, having molecular weight of 2700 isolated from the urine of normal person.

The studies presented in the dissertation thus clearly indicate that both quantitative and qualitative difference exist in the urine of normal persons and kidney stone patients regarding the biomolecules which can influence mineralization and demineralization reactions. The differences observed here may lead to a better understanding of the etiology of renal stone formation at the molecular level.