when using a quantitative assay procedure. Ascorbic acid appeared to degrade to a greater extent in Emulsion 1 than in Emulsion 2. This finding agreed with the results of Bandelin and Tuschhoff (17), who found that vegetable gums added to produce liquids of greater viscosity seemed to accelerate the degradation of ascorbic acid. However, the main point to be emphasized once again is that drug degradation within a stable emulsion can be monitored at least semiquantitatively by DRS. Using DRS alone with no quantitative assay, one could conclude with little trepidation that ascorbic acid was unstable in these emulsion systems.

Figure 8 is shown to test the Kubelka–Munk hypothesis relating $f(r_\infty)$ directly to the concentration of the absorbing species. A linear relationship was evident for the drug in Emulsion 2 but not for the drug in Emulsion 1. Unfortunately for this drug emulsion system, no other wavelength could be used as an indicator of ascorbic acid degradation. However, the evidence indicates that ascorbic acid degradation in an emulsified formulation was monitored adequately by DRS.

SUMMARY AND CONCLUSIONS

DRS is a simple method and can be used in combination with other methods for evaluating emulsion stability. The method is sensitive and can detect subtle changes in the emulsion microenvironment, but it does not demand sampling or manipulation of the system. Should a semiquantitative estimate of an active component within an emulsion be desired, then the DRS method offers the unique advantage of estimating drug stability without disturbing the system.

Additional research is recommended to improve or define exactly the interpretation of the data obtained from reflectance measurements. These studies only show the application of DRS as a corroborative tool for the monitoring and evaluation of emulsion stability and drug stability within an emulsified system.

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Retardation of Dissolution and Growth of Calcium Oxalate Monohydrate

S. BISAILLON and R. TAWASHI [x]

Abstract □ The kinetics of dissolution and growth of calcium oxalate monohydrate were examined in the presence of small concentrations of pyrophosphate, chlorophyll, and other agents. Data presented show that the retardation in mass transport in both processes is controlled by the nature of the additive, its concentration, and the way the additives are combined in the dissolution medium. Dissolution was studied using a particle counter method, and growth was conducted in a gel system under the slow diffusion of the reacting ions. Results obtained show that chlorophyll is more active than other inhibitors studied and suggest a higher surface adsorption intensity on the primary sources of the crystal surface.

Keyphrases □ Calcium oxalate monohydrate—kinetics of dissolution and crystal growth, effects of various additives alone and in combination □ Dissolution—calcium oxalate monohydrate, kinetics, effects of various additives alone and in combination □ Crystal growth—calcium oxalate monohydrate, kinetics, effects of various additives alone and in combination □ Renal stone formation—calcium oxalate monohydrate, kinetics of dissolution and crystal growth, effects of various additives alone and in combination

Genesis and growth of calcium oxalate calculi are still unclear (1). To explain the ability of urine to remain supersaturated without precipitation, crystallization inhibitors should be studied. Equally important are the factors influencing the kinetics of dissolution of the stone components.

The complexity of urine in stone formation is certainly responsible for the slow progress in the area. The interaction of one compound with the others [*e.g.*, the action of magnesium on the solubility of calcium oxalate (2)] and the presence of material with a high epitaxial activity [such as organic material (3–

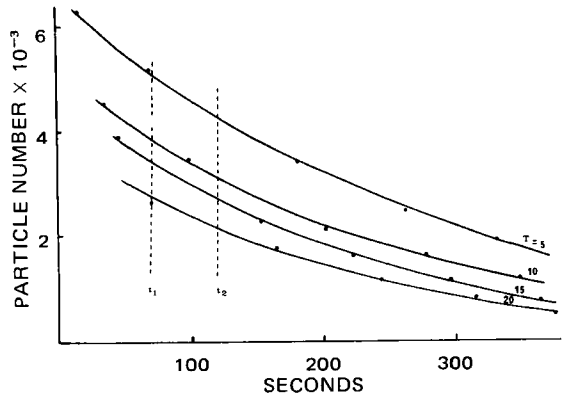

Figure 1—*Dissolution rate determination method showing the counts of particles as a function of time for different threshold settings* (T).

5)] make the clinical picture an undesirable approach in understanding basically renal stone formation.

A previous report from this laboratory, dealing with the influence of pH and magnesium on dissolution and growth rates of calcium oxalate monohydrate, demonstrated the effect of these parameters on the kinetics of these processes (6). Intensive and controversial studies were reported on the effect of pyrophosphate as a potential inhibitor of calcium oxalate growth (7–9), and no data are available on the dissolution process. Recent work demonstrated the possibility of using a gel technique to grow artificial concretions of calcium oxalate (10) and calcium carbonate (11).

The present work examines the effect of pyrophosphate, chlorophyll, and other agents on both processes. Quantitative data on the influence of these additives, alone and in combination, are presented, and the mechanism of action on the crystal surface is discussed.

EXPERIMENTAL

Materials—The following materials were used: calcium chloride dihydrate, oxalic acid dihydrate, sodium pyrophosphate decahydrate, potassium chloride, potassium carbonate, sodium orthophosphate, magnesium chloride, hydrochloric acid, and methylene blue (all reagent grade). Tromethamine[1], gelatin[2], chlorophyll[3], povidone[4], and normal saline[5] also were used.

Dissolution Rate Studies—The dissolution rate determinations were carried out using the particle counter[6] technique described previously (6). A suspension of calcium oxalate monohydrate was added to saline solution buffered at pH 6.2. A series of counts was taken, and cumulative size distribution curves were plotted. From the change in diameter for a fixed number of particles, the rate of dissolution was determined for a selected time period (t_1 and t_2) (Fig. 1).

The data were manipulated as previously described, and the dissolution rates were determined in centimeters per second in the absence and presence of additives at different levels of concentration. The estimated error by this method was approximately 5%.

Growth Studies—Growth studies were carried out using a gel technique (12). Figure 2 illustrates the U-tube system used for growing calcium oxalate crystals. In the U-shaped tube, each ion diffused from each branch of the tube; crystallization occurred at a front determined by the diffusion speed of each ion species. Figure 2 also illustrates the concentration variation as a function of distance in the tube for both reacting ions. The critical concentration of the precipitating components obeys Fick's second law of diffusion, as demonstrated by Pučar *et al.* (13).

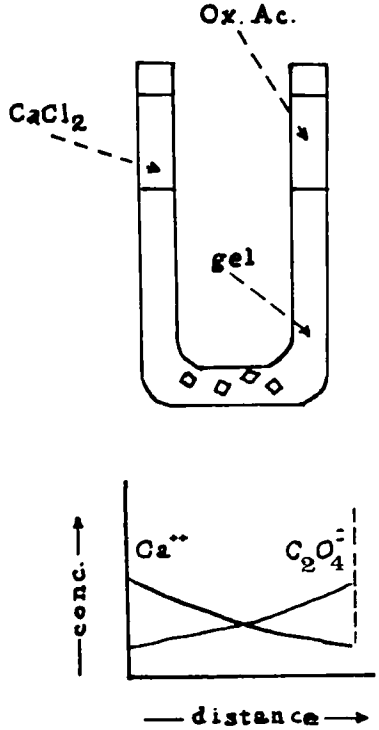

Figure 2—(*Top*) *U-tube system used for growing calcium oxalate crystals.* (*Bottom*) *Concentration variation as a function of distance for both ions.*

The growth support medium used was a gelatin gel in which the additive was incorporated. The gelatin was preferred to silica for its high purity regarding the presence of other ions (14). It also has a high optical clarity for crystal observations, and quantitative recovery does not present any difficulty. Gelatin also has the advantage of being an organic material and close to collagen in its structure, properties that can lead to interesting conclusions.

A 5% aqueous gelatin solution was prepared by gentle heating (below 50°) for 1 hr. Formaldehyde solution was added to prevent mold growth at a concentration of 0.1%. The solution was buffered to pH 6.2 using tromethamine. Then 25 ml of the solution was poured into the U-tubes and allowed to gel for 24 hr in a controlled-temperature room (22°). When additives were studied, 1 ml of the additive in aqueous solution was added prior to gelling to obtain the desired concentration.

After 24 hr, 10 ml of calcium chloride (0.5 *M*) and oxalic acid (0.5 *M*) solutions were layered carefully on top of the gel on each

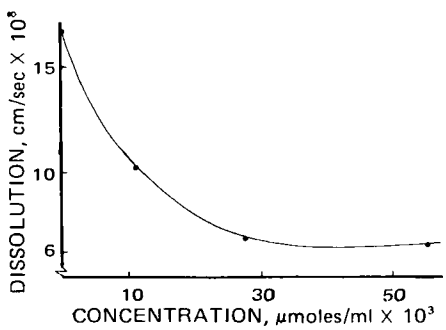

Figure 3—*Dissolution rate of calcium oxalate monohydrate in the presence of small concentrations of chlorophyll.*

[1] THAM, Fisher Scientific Co., Montreal, Canada.
[2] Purified calfskin, Eastman Kodak Co., Rochester, N.Y.
[3] Chlorophyllin copper complex sodium salt, E. Merck Chemicals; distributed by Brinkmann Instruments, Rexdale, Canada.
[4] Kollidon 17, B.A.S.F. Chemicals, Canada Ltd., Montreal, Canada.
[5] Sodium chloride injection USP, Abbott Laboratories, Montreal, Canada.
[6] Coulter.

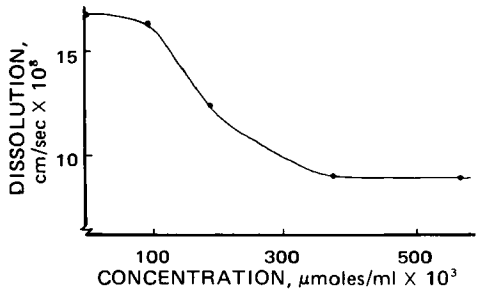

Figure 4—*Dissolution rate of calcium oxalate monohydrate in the presence of small concentrations of pyrophosphate.*

side of the tubes. After incubation at 22° for a fixed period, the crystals were examined in polarized light and recovered quantitatively. The recovery was achieved by centrifugation of the melted section containing the crystallization front and separation of the supernate.

Several washings were needed to discard gelatin or the other foreign additives from the calcium oxalate crystals. Maximum crystal yield was obtained after 6 days. In these experiments, the estimated error was approximately 8%.

RESULTS AND DISCUSSION

The dissolution rate dependence on additive concentration was systematically determined. Figure 3 shows the rate of dissolution of calcium oxalate monohydrate as a function of chlorophyll concentration at pH 6.2. This plot demonstrates the remarkable effect of chlorophyll as a powerful inhibitor of dissolution. A 2.5-fold decrease in the dissolution rate was reached with a concentration as low as 27.55×10^{-3} μmole/ml. Pyrophosphate demonstrated a lower activity than chlorophyll (Fig. 4), and the dissolution rate tended to stabilize at 37.59×10^{-2} μmole/ml. Methylene blue showed dissolution inhibition approximately similar to pyrophosphate for about the same concentration (20.06×10^{-2} μmole/ml).

It was interesting to observe that povidone acted differently on the dissolution rate of calcium oxalate monohydrate. An increase in the dissolution rate (25%) was noticed at a concentration of 29.41×10^{-4} μmole/ml. Further study is needed to explain the mechanism behind the increase in the mass transport of calcium oxalate in the presence of povidone. Other tested substances like potassium chloride, potassium carbonate, and sodium orthophosphate gave less significant inhibition even at higher concentrations. Table I summarizes the data obtained for other additives.

The remarkable inhibiting activity observed with chlorophyll and pyrophosphate at low concentration encouraged the examination of the effect of these additives in combination with other anions and cations with the aim of potentiating their inhibitory effect. Chlorophyll, at a concentration of 0.01 μmole/ml, was combined with small concentrations of potassium, orthophosphate, and magnesium [the latter demonstrated no effect on the dissolution rate as reported previously (6)] and with another active substance, pyrophosphate. Combining two additives improved the dissolution inhibition activity (Fig. 5). In Fig. 5, for example, the second diagram shows the combination effect of chlorophyll and potassium on the dissolution rate. The first column represents the action of chlorophyll alone, the second represents the action of potassium alone, and the third represents the effect of a combination of chlorophyll and potassium.

Table I—Dissolution Rate Values for Calcium Oxalate Monohydrate in the Absence or Presence of Different Additives

	Concentration, μmoles/ml	Dissolution Rate, cm/sec × 10^8
No additive	—	16.8
Methylene blue	0.134	10.0
Povidone	0.003	21.0
Chlorophyll	0.055	6.5
Pyrophosphate	0.188	12.4
Orthophosphate	3.0	8.5
Potassium		
As chloride	3.0	12.5
As carbonate	3.0	16.5

Table II—Yield of Calcium Oxalate Crystallites Grown in Gelatin Gel in the Absence or Presence of Different Additives after 3 Days of Incubation

Additive	Quantities Recovered from the Gel, mg
None	24.3
Chlorophyll	10.0
Pyrophosphate	8.4
Magnesium	7.4

Again, the combination of the two most active inhibitors in this study, chlorophyll and pyrophosphate, with an ionic substance gave a more pronounced effect on the dissolution rate (Fig. 6). The dissolution rate of calcium oxalate was reduced six fold in the presence of chlorophyll–pyrophosphate–magnesium in the dissolution medium.

The growth rate studies using the gelatin gel medium resulted in a significant reduction of the yield of calcium oxalate crystallites (Table II). The addition of chlorophyll, pyrophosphate, and magnesium lowered the quantity of crystals separated from the gel growth front by 2.5–3-fold after 3 days of incubation. The results obtained with magnesium are in agreement with previous data using the particle counter method (6). Other tested additives are presently being evaluated.

The present findings show that chlorophyll and pyrophosphate exert a remarkable inhibition on the dissolution and growth of calcium oxalate crystals. This inhibition, as far as the dissolution is concerned, is much more pronounced when pyrophosphate and/or chlorophyll are combined with an ionic substance. The effect observed cannot be attributed to a change in ionic strength, since the concentrations added (3.0 μmoles/ml) in the dissolution medium would not change this parameter significantly (the dissolution medium being 0.9% saline solution).

The inhibition produced by pyrophosphate, chlorophyll, and methylene blue can be examined in the light of the Langmuir ad-

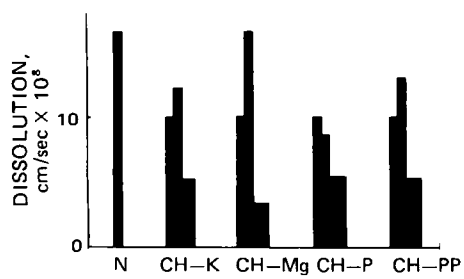

Figure 5—*Histogram showing the effect of combining chlorophyll (CH, 0.01 μmole/ml) with other additives. Key: N, no additive; PP, pyrophosphate (0.11 μmole/ml); K, potassium (3.0 μmoles/ml); Mg, magnesium (3.0 μmoles/ml); and P, orthophosphate (3.0 μmoles/ml). (See text.)*

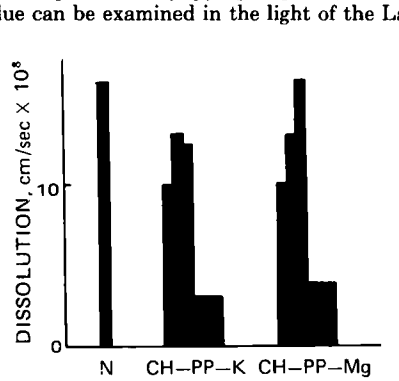

Figure 6—*Histogram showing the effect of combining chlorophyll (CH, 0.01 μmole/ml) and pyrophosphate (PP, 0.11 μmole/ml) with other additives. Key: N, no additive; Mg, magnesium (3.0 μmoles/ml); and K, potassium (3.0 μmoles/ml).*

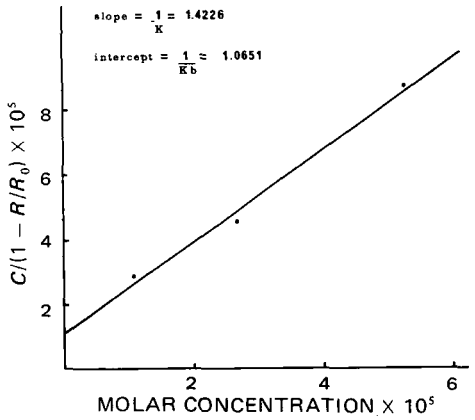

Figure 7—*Determination of adsorption constant for calcium oxalate monohydrate in the presence of chlorophyll according to Langmuir adsorption.*

sorption isotherm. If it is assumed that: (*a*) the fractional rate reduction caused by the additive is proportional to the fraction of the surface covered by the inhibitor, and (*b*) the adsorption of the substance can be represented by the simple Langmuir adsorption isotherm, the dissolution rate of calcium oxalate can be expressed as a simple function of the inhibitor concentration in solution as:

$$R = R_0\left[1 - \frac{KbC}{1 + bC}\right] \qquad \text{(Eq. 1)}$$

where R = dissolution rate of calcium oxalate monohydrate with one inhibitor, R_0 = dissolution rate of calcium oxalate monohydrate without additive, C = concentration of the inhibitor in the bulk solution, K = fraction of the surface covered when the surface is saturated with the inhibitor, and b = Langmuir adsorption constant reflecting the affinity of the substance for the binding sites on the crystal surface.

Equation 1 was rearranged and $C/(1 - R/R_0)$ was plotted against C, using a linear regression program for each substance studied. An example of the plots obtained can be seen in Fig. 7 for chlorophyll. From these data, a correlation with the Langmuir adsorption isotherm can be established. The values of the constants K and b were determined from the slopes and intercepts, respectively (Table III).

The K value, a measure of the saturation capacity, is approximately the same for pyrophosphate and chlorophyll; however, the b value, illustrating the affinity of the bound substance on the crystal surface, is 30 times greater for chlorophyll than for pyrophosphate. This finding explains the higher dissolution inhibition obtained with chlorophyll than with pyrophosphate. For methylene blue, which exerts an inhibition activity approximately the same as pyrophosphate, the surface coverage (K value) is smaller than for pyrophosphate; but its surface adsorption intensity on the crystal, as represented by the b value, is much more pronounced.

Table III—Values of the Constants K and b for the Langmuir Adsorption Isotherm Correlation[a]

	K	b
Chlorophyll	0.7029	1.3357
Pyrophosphate	0.7505	0.0457
Methylene blue	0.4189	0.7492

[a] K represents the fraction of the surface covered when the surface is saturated with the inhibitor, and b represents the Langmuir adsorption constant related with the affinity of the substance (strength of the bonds) for the surface.

The growth retardation in the presence of chlorophyll, pyrophosphate, and magnesium in the gel system can be explained in the same manner; more studies are necessary for complete characterization of the effect of these additives on crystal morphology.

The present observations and results show that very small changes in the levels of these factors in the biological system, for a relatively short period, can change the whole pattern of deposition and dissolution of calcium oxalate.

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