

AMORPHOUS CALCIUM PHOSPHATE PRECIPITATION IN AQUEOUS SUPERSATURATED SOLUTIONS: THE INFLUENCE OF NATURAL AND SYNTHETIC INHIBITORS

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**Abstract** The induction time for the transformation reaction of amorphous calcium phosphate (ACP) to hydroxyapatite (HAP) was investigated at pH 7.40, 37 °C, and calcium = phosphate 3.20 mM, in the presence of natural and synthetic inhibitors containing phenolic and carboxyl groups. The inhibitors tested include: natural materials containing phenolic and carboxyl groups i.e., green tea extract, GT; lignosulfonate, LS; fulvic acid, FA; tannic acid, TA; and synthetic material containing carboxyl group i.e., polyacrylic acid. It has been found that all inhibitors were effective in delaying the transformation reaction. The inhibitor effectiveness was greatly affected by inhibitor concentration and the presence of functional groups i.e., phenolic and carboxyl. For natural and synthetic inhibitors the order in terms of decreasing effectiveness is: PA >> FA > TA > GT > LS.

INTRODUCTION

Precipitation of calcium phosphates poses serious challenges in biological systems. Common examples include dental calculus, calcified plaques arteriosclerosis, kidney calcinosis, etc. Calcium phosphates deposits have been encountered on heat exchangers and during pasteurization of milk. In addition, calcium phosphates are widely produced in industry, in such forms as ceramics, nutrient supplements, medicine, dentrifices, and stabilizer for plastics.

Hydroxyapatite ( $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ , HAP) is known as the main component of hard tissues i.e., bones and teeth. At low supersaturation, HAP directly crystallizes and grows in solution. On the other hand, at high supersaturation it is generally agreed that the initially precipitated phase, at pH above 7, is an amorphous calcium phosphate, ACP. The mechanism of the precipitation of ACP from solution has not yet been fully established. Crystallization of HAP in biological and industrial systems is affected by many kinds of inhibitors, which play an important role in the regulation of many minerals deposition. The influence of various inhibitors on the precipitation of calcium phosphate under physiological conditions has been the subject of several investigations. Results of these studies indicate that inhibitors such as polyphosphates, magnesium, citric acid, adenosine triphosphate and polycarboxylic acids when present at low concentrations affect the transformation of ACP to HAP<sup>1-3</sup>.

During the last two decades dramatic advances have occurred in preventing the precipitation and deposition of calcium phosphate on various substrates i.e., heat exchanger, semi-permeable membrane, tooth enamel, and equipment surfaces<sup>4</sup>. As a

result, synthetic polymers are finding increased application in water treatment. Imai et al.<sup>5</sup>, Amjad<sup>6,7</sup> and Amjad et al.<sup>8</sup> in their studies on the evaluation of polymers of varying compositions concluded that both the functional group and ionic charge of the polymer play important roles in imparting inhibitory activity to polymers. For carboxylic acid containing polymers, it appears that precipitation inhibition is greatest for molecular weight of less than 20,000, with the optimum molecular weight being dependent on the particular polymer and scale forming salt being formed.

Natural organic compounds (i.e., humic substances) are commonly encountered in surface water and ground water used for water supply. Humic substances are mixtures of natural products with a complex structure and chemistry. They mostly contain carboxyl and phenolic functionalities, and can behave as negatively charged colloids or anionic polyelectrolytes in natural waters. Several studies have been reported pertaining to the influence of natural organic compounds on the precipitation of various scale forming salts. Results of these studies reveal that low levels of fulvic acid markedly inhibit the crystal growth of calcium phosphate and calcium carbonate salts<sup>9,10</sup>. Lignosulfonates are classified as anionic polyelectrolytes and can vary in molecular weights. The derivatives used as dispersant and crystal modifiers are in the 1,000 to 20,000 mole weight range. Lignosulfonates vary widely in nature partly due to variation in the original source and type of wood.

It is generally accepted that chronic exposure of solar UV radiation to human skin induces a number of pathologic conditions including erythema, sunburn cell formation, DNA damage, photoaging and skin cancer development. Abundant available information implicates the involvement of UV-induced inflammatory responses and reactive oxygen species or oxidative stress in these pathologic conditions. Recently, the use of botanicals in skin care products has attracted the attention of several researchers, cosmetic chemists, and consumers. In addition, in studies involving the chronic oral feeding of green tea to hairless mice followed by irradiation with UV, was found to result in significant protection against UV-induced skin diseases<sup>11</sup>.

To understand the interaction of phenolic groups present in GT we studied the transformation reaction of amorphous calcium phosphate (ACP) to hydroxyapatite under physiological conditions. This study also presents results on the effect of various natural phenolic compounds i.e., lignosulfonate, fulvic acid, and tannic acid. The influence of these natural polyphenolic compounds are compared with synthetic polycarboxylic acid material.

## EXPERIMENTAL

Reagent grade chemicals, grade A glassware, and CO<sub>2</sub>-free distilled water were used. Stock solutions of potassium hydrogen phosphate and calcium chloride were prepared using distilled water and analyzed as described previously<sup>8</sup>. The inhibitors used were selected from commercial materials. Inhibitors used in the present study were obtained as follows: Green tea extract, GT, (30 % phenolic, Carrubba, Inc.), lignosulfonate (Water Engineering Services, Phoenix, AZ), fulvic acid, FA (Dr. M. Reddy, U.S. G. S., Boulder,

CO), and tannic acid, TA (Fisher Scientific). Poly(acrylic acid) used was obtained from Noveon, Inc., Cleveland, OH. All inhibitors solutions were prepared on a dry weight basis. The desired concentrations were obtained by dilution. All experiments were performed in a covered water-jacketed cell of 250 mL maintained at 37 °C.

Subsaturated solutions were prepared by placing known volumes of calcium chloride and potassium hydrogen phosphate. The total volume of the calcium phosphate solution was 220 mL. Spontaneously precipitation of calcium phosphate solution was induced by raising the pH of the acidic solution with 0.10 M NaOH solution. The pH of the solution was maintained constant ( $7.40 \pm 0.01$ ) using pH-stat apparatus as described previously<sup>8</sup>. Hydrogen ion measurements were made with a combination electrode equilibrated at the required temperature. The calcium phosphate solutions were continuously stirred with a Teflon<sup>®</sup> coated stirring bar at ~ 350 revolutions per minute and nitrogen gas pre-saturated at 37 °C was bubbled to exclude carbon dioxide from the solution. Experiments involving inhibitors were performed by adding the inhibitor to the subsaturated calcium phosphate solution before inducing spontaneous precipitation. The induction time for ACP to HAP transformation reaction was calculated according to the procedure described previously<sup>12</sup>.

## RESULTS AND DISCUSSION

Table 1 summarizes the results on the ACP to HAP transformation reaction in the presence of varying concentrations of inhibitors. Typical plots of the amount of NaOH added to the calcium phosphate solution to maintain constant pH are shown in Figure 1. The data in Figure 1 was used to determine the induction time,  $\beta$ , for the transformation of ACP to HAP phase. This was determined as illustrated by the arrow in Figure 1. The performance of GT as ACP inhibitor is presented in Figure 1. It can be seen that inhibitor performance depends on the concentration of GT present in calcium phosphate solution. As illustrated in Figure 1 the  $\beta$  value obtained in the presence of 75 ppm of GT is 3 minutes compared 2 minutes obtained in the absence of GT thus suggesting that GT at 75 ppm concentration does not significantly affect the induction time. However, as illustrated in Figure 1 increasing the concentration of GT from 100 ppm to 250 ppm, results in ~ 250 % increase in induction time i.e., from 8 min to 20 min.

The results of experiments to compare the performance of lignosulfonate (LS) as ACP inhibitor are summarized in Table 1 and illustrated in Figure 2. It is evident from Figure 2 that LS is not an effective inhibitor for ACP. For example, the  $\beta$  values obtained in the presence of 100 and 250 ppm of LS are 3 and 7 min respectively, compared to 8 and 20 min obtained in the presence of 100 and 250 ppm of GT. The difference in inhibitor performance between two natural polyphenolic inhibitors may be attributed to various factors including number of phenolic groups, molecular weight, and impurities. It is interesting to note that similar trend in inhibitor performance has been observed for the precipitation of calcium carbonate from aqueous solution<sup>13</sup>.

In order to compare the performance of natural inhibitors containing both phenolic and carboxylic acid groups, a series of ACP precipitation experiments were carried out in the

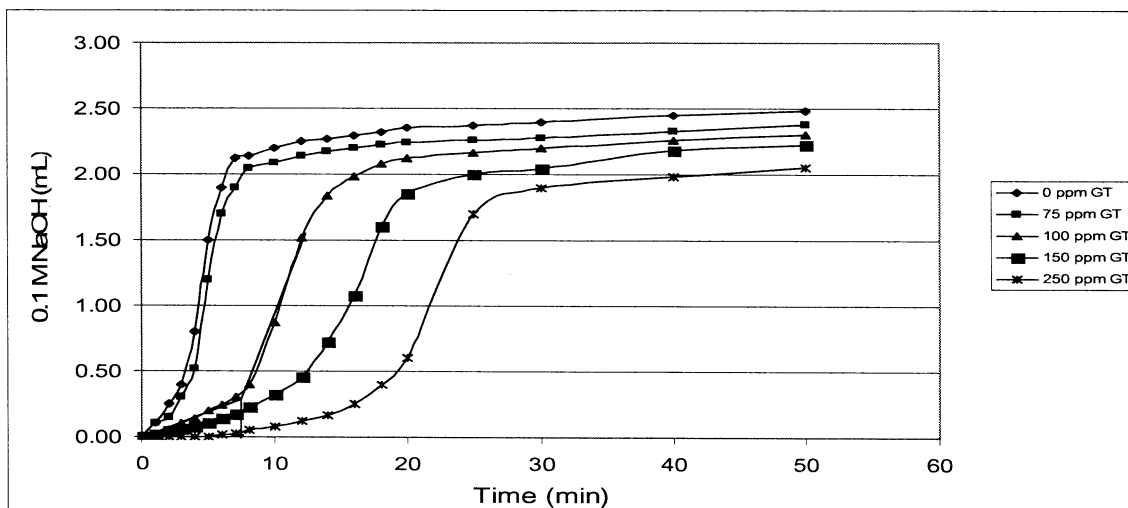


FIGURE 1. NaOH uptake as a function of time in the presence of GT

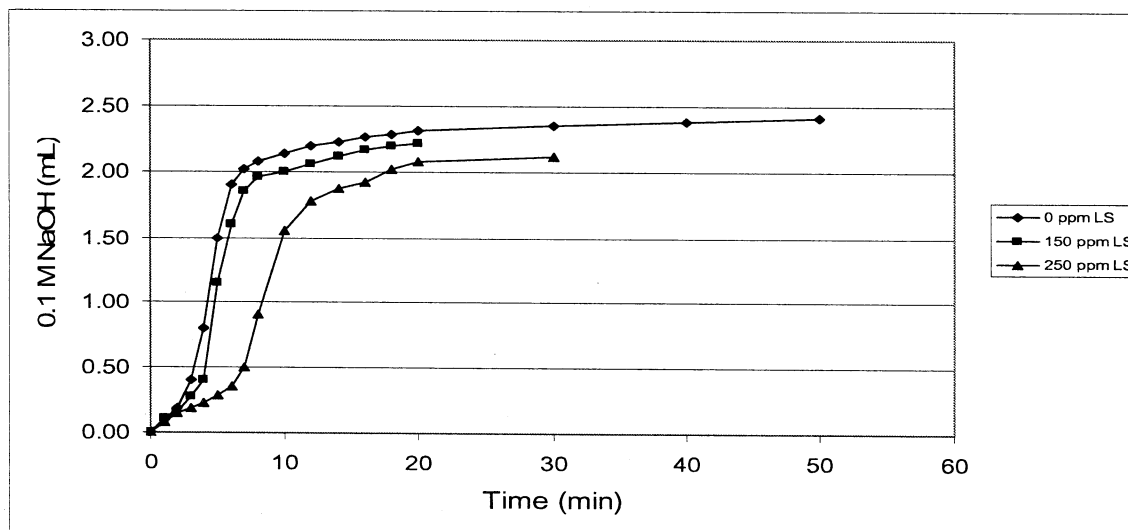


FIGURE 2. NaOH uptake as a function of time in the presence of LS.

presence of fulvic acid (FA) and tannic acid (TA). Results of these experiments are summarized in Table 1 and illustrated in Figure 3. It can be seen that both FA and TA are effective ACP inhibitors. For example, the  $\beta$  values obtained (Table 1) in the presence of 50 ppm of inhibitors are 11 and 8 min for FA and TA, respectively. On the basis of induction values, the order in terms of decreasing effectiveness is as follows: FA > TA >> GT > LS. The observed order of inhibitor effectiveness may be attributed to the presence of carboxylic acid group in addition to phenolic groups in FA and TA.

The assessment of the effectiveness of the synthetic polycarboxylic acid i.e., poly(acrylic acid), PA, as ACP inhibitor was done by carrying out a series of precipitation experiments in the presence of varying concentration of PA (MW 6,000). Figure 3 shows NaOH uptake – time profiles in the presence of 50 ppm of PA. As can be

seen PA at low concentration has a pronounced effect on the induction time. For example,  $\beta$  values obtained in the presence of 25 and 50 ppm of PA are 5 and 21 min, respectively, compared to 2 min obtained in the absence of PA. Based on the data presented in Table 1 the inhibitors tested in this study can be ranked in terms of decreasing effectiveness as follows: PA >> FA > TA >>GT > LS.

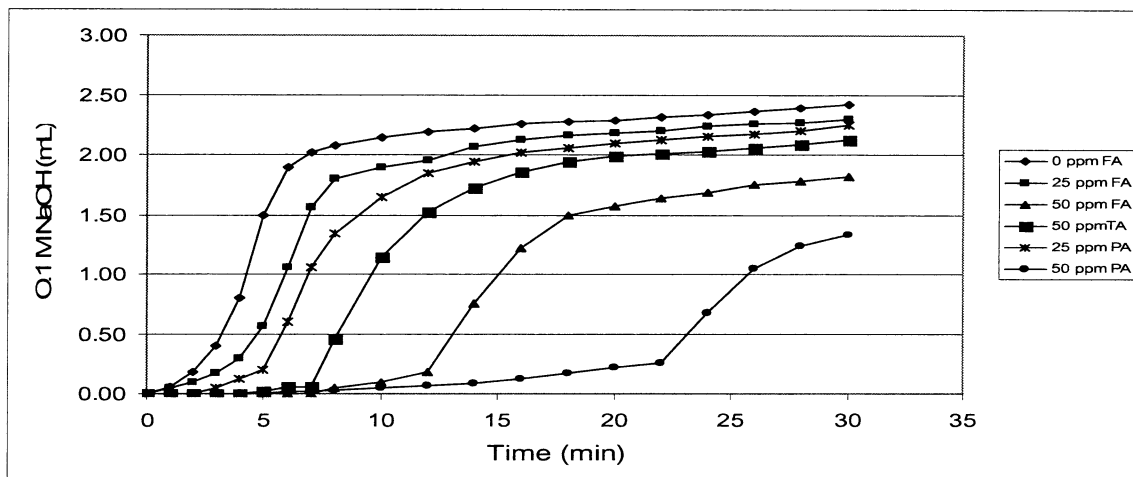


FIGURE 3. NaOH uptake as a function of time in the presence of FA, TA, and PA.

TABLE 1. Induction times for the ACP precipitation in the presence of natural and synthetic inhibitors

Exp No.	Inhibitor	Concentration (ppm)	Induction time (minutes) ( $\beta$ )
1.	None	0.0	2
2.	GT	50	2
3.	GT	75	3
4.	GT	100	8
5.	GT	150	12
6.	GT	250	20
7.	SL	100	3
8.	SL	150	4
9.	SL	250	7
10.	FA	25	4
11.	FA	50	11
12.	TA	25	3
13.	TA	50	8
14.	PA	25	5
15.	PA	50	21

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