Adsorption on and Surface Chemistry of Hydroxyapatite

Edited by Dwarika N. Misra

Adsorption on and Surface Chemistry of Hydroxyapatite

Edited by
Dwarika N. Misra
America Dental Association Health Foundation
Research Unit, National Bureau of Standards
Washington, D.C.

Plenum Press • New York and London

Library of Congress Cataloging in Publication Data

Symposium on Adsorption on and Surface Chemistry of Hydroxyapatite (1982: Kansas City, Mo.)
Adsorption on and surface chemistry of hydroxyapatite.

"Based on proceedings of the Symposium on Adsorption on and Surface Chemistry of Hydroxyapatite, held September 12–17, 1982, at the ACS meeting in Kansas City, Missouri"—T.p. verso.
Includes bibliographical references and index.
1. Hydroxyapatite—Congresses. 2. Adsorption—Congresses. 3. Surface chemistry—Congresses. I. Misra, Dwarika N. II. American Chemical Society. III. Title.
QD181.P1S96 1982 546'.71224 83-24738
ISBN 0-306-41556-X

Based on Proceedings of the Symposium on Adsorption on and Surface Chemistry of Hydroxyapatite, held September 12-17, 1982, at the ACS meeting in Kansas City, Missouri

A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

Printed in the United States of America

Adsorption on and Surface Chemistry of Hydroxyapatite

PREFACE

Hydroxyapatite is the structural prototype of the main inorganic constituent of bone and teeth and, together with fluorapatite, is also one of the principal minerals in commercial phosphate ores. The adsorption characteristics and surface chemistry of hydroxyapatite are important in understanding the growth, dissolution and adhesion mechanisms of bone and tooth tissues and in elucidating the factors in mineral beneficiation such as floation and flocculation.

This volume essentially documents the proceedings of the symposium on the same topic held at the American Chemical Society Meeting in Kansas City, MO, September 12-17, 1982. It includes a few papers which were not presented at the symposium but does not comprise the entire program.

This volume provides, on a limited scale, a multidisciplinary overview of current work in the field of adsorptive behavior and surface chemistry of hydroxyapatite and includes certain review articles. There are two papers each on adsorption, adsorption and its effects on crystal growth or dissolution kinetics, effects of electrochemical parameters on solubility and adsorption, and newer physical methods (exoemission and high-resolution NMR) of examining hydroxyapatite surface. There is one paper each on structure modelling of apatite surface based on octacalcium phosphate interface and on biodegradation of sintered hydroxyapatite.

I wish to express my appreciation to the management of the American Dental Association Health Foundation for permitting me to organize the symposium and to edit this volume, acknowledging primarily the understanding and patience of Drs. R. L. Bowen and W. E. Brown. I thank the reviewers for their time and valuable comments and the authors for their enthusiastic cooperation. I am

particularly grateful to Mrs. L. E. Setz for her prompt and competent secretarial services throughout the project period, and I especially thank my wife, Chandra, for her cooperation and forbearance during the same period.

August, 1983
Gaithersburg, MD

Dwarika N. Misra

CONTENTS

THE INFLUENCE OF MELLITIC ACID ON THE CRYSTAL GROWTH OF HYDROXYAPATITE

Zahid Amjad

BFGoodrich Chemical Group
Avon Lake Technical Center
P. O. Box 122
Avon Lake, OH 44012

ABSTRACT

The constant composition method has been used to study the influence of mellitic acid (benzene hexacarboxylic acid) on the kinetics of crystal growth of hydroxyapatite (HAP) at low constant supersaturation. Addition of mellitic acid to the calcium phosphate supersaturated solutions has a striking inhibitory influence upon the rate of crystal growth of HAP. The effect is interpreted in terms of adsorption, following the Langmuir isotherm, of mellitate ions at the active crystal growth sites.

INTRODUCTION

The mineralization of calcium phosphates, which is present in biological hard tissues, is complicated by the existence of four phases namely: dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$); octacalcium phosphate ($Ca_8H_2(PO_4)_6 \cdot 5H_2O$,OCP); tricalcium phosphate ($Ca_3(PO_4)_2$, TCP); and hydroxyapatite ($Ca_5(PO_4)_3OH$, HAP). Results of previous studies[1-4] on the spontaneous precipitation of calcium phosphates at physiological conditions indicate that the kinetically favored precursor phases are formed prior to the formation of the thermodynamically stable HAP. Moreover, it has also been suggested that such precursor phases can be stabilized by the presence of certain additives.[5,6]

The influence of naturally occurring substances such as pyrophosphate and polyphosphates, isolated from serum and urine, on the precipitation of calcium phosphates at physiological conditions has also been the subject of numerous investigations.[7-9]

The results of these studies suggest that these substances when present at low concentrations greatly inhibit the precipitation of calcium phosphates from supersaturated solutions. With the rather limited hydrolytic stability of the pyrophosphate ion, structurally related phosphonate compounds containing P-C-P bond in place of P-O-P bond, have been developed and their inhibitory activities on the crystal growth of calcium oxalate,[10] calcium sulfate,[11,12] barium sulfate,[13] calcium carbonate,[14-16] and calcium phosphates,[17-19] have been investigated by many workers.

The inhibitory effect of polycarboxylic acids on the precipitation of calcium phosphates has also been recently studied. Comparison of the inhibitory activity of citric acid, isocitric acid, and tricarballylic acid on calcium phosphate crystallization at physiological conditions using seeded growth technique, suggests that the hydroxyl group in the molecular backbone is a key factor in the effectiveness of these tricarboxylic acids as inhibitors.[20]

The influence of mellitic acid (benzene hexacarboxylic acid, MA) and phosphonate (hydroxyethylidene-1, 1-diphosphonic acid, EHDP) on the precipitation of calcium phosphate from solutions supersaturated with respect to all calcium phosphate phases, has also been recently investigated.[21] The results of this study indicate that although MA protects the enamel surface in acid solution and appears to have inhibiting properties similar to EHDP, EHDP is more effective as an anticalculus agent compared to MA.

Results of recent studies[18,22-25] have shown that kinetics of crystal growth of HAP as exclusive phase on different seed materials can be accurately studied by constant composition technique. Because of the recent interest in the inhibitory effect of polycarboxylic acids on the crystallization of calcium phosphates, in the present work the constant composition method has been extended to investigate the influence of mellitic acid on the crystal growth of hydroxyapatite at low sustained supersaturation.

EXPERIMENTAL

Grade A glassware and reagent grade chemicals were used. Phosphate stock solutions were made from potassium dihydrogen phosphate and were standardized potentiometrically by titration with standard potassium hydroxide (Dil-It, J. T. Baker Co.). Calcium stock solutions were prepared from recrystallized calcium chloride dihydrate (J. T. Baker Co.) and were standardized by ion exchange method (Dowex-50W-X8) and atomic absorption spectroscopy. Mellitic acid solutions were prepared from mellitic acid (Aldrich Chemical Co.). The HAP seed crystals, prepared and characterized

as previously described,[26] were aged for at least five months before use. The specific surface area determined by a single point BET method using a N_2/He-30:70 gas mixture for the HAP seed crystals was $35.8 m^2 g^{-1}$.

Crystal growth experiments were made in a double-walled Pyrex cell at 37C using the constant composition technique.[27] The stable supersaturated solutions of calcium phosphate with a molar ratio of Tc_a: Tp=1.67, were prepared by adjusting the pH of a premixed subsaturated solution of calcium chloride and potassium dihydrogen phosphate to a value of 7.40 by slow addition of 0.1M potassium hydroxide. pH measurements were made with a glass/calomel electrode pair equilibrated at 37C. The electrode pair was standardized before and after each experiment using NBS standard buffer solutions.[28] The solutions were continuously stirred (300 rpm) while nitrogen gas, presaturated with water at 37C, was bubbled through the solution to exclude carbon dioxide. Following the inocculation with HAP seed crystals, the crystal growth reaction was monitored by the addition of titrant solutions from mechanically-coupled automatic buretts mounted on a modified[27] pH-stat (Metrohm-Herisau, Model 3D combititrator, Brinkmann Instruments, Westbury, N.Y.). The titrant solutions in the buretts consisted of calcium chloride, potassium phosphate, potassium hydroxide, and mellitic acid. The molar concentration ratio of the titrant corresponded to the stoichiometry of the HAP phase. Potassium chloride was added to the calcium phosphate supersaturated solutions in order to maintain the ionic strength to within 1%. The constancy of solution composition was verified by analyzing the filtered samples which were withdrawn at various time intervals, for calcium and phosphate according to method described previously.[29] The rates of crystallization were determined from the rates of addition of mixed titrants, and corrected for surface area changes.[30]

RESULTS AND DISCUSSION

The computation of concentrations of ionic species were made, from mass balance, electroneutrality, proton dissociation, and equilibrium constants involving calcium ions with mellitic acid, by iterative procedure as described previously.[31] Published values for phosphoric acid, mellitic acid dissociation constants,[32] calcium phosphate ion-pair association constant and calcium mellitate equilibrium constants,[33] used in these calculations are shown in Table I. The free energy term is computed from the equation

$$\Delta G = - RT \ln IP/K_{so} \qquad (1)$$

in which IP is the ionic activity product and K_{so}, the value of IP at equilibrium. R and T are the ideal gas constant and absolute temperature. The ΔG values obtained by using the

TABLE I

CRYSTALLIZATION OF HAP ON HAP SEED CRYSTALS IN THE PRESENCE OF MELLITIC ACID[a]

Exp #	MA x 10^{-6}M	ΔG_{DCPD}*	ΔG_{OCP}*	ΔG_{TCP}*	ΔG_{HAP}*	Rate x 10^{8}**
16	0.0	+4.03	+1.58	-9.94	-52.4	11.2
15	0.160	+4.03	+1.58	-9.94	-52.4	9.08
13	0.250	+4.03	+1.58	-9.94	-52.4	8.04
10	0.400	+4.03	+1.58	-9.94	-52.4	6.79
14	1.00	+4.03	+1.58	-9.93	-52.3	4.33
12	3.00	+4.04	+1.59	-9.92	-52.3	1.94

* (kJ $mole^{-1}$)
** (mole $min^{-1}m^{-2}$)

Note: Initial Conditions: T_{Ca}=0.500 x 10^{-3}M, Tp=0.300 x 10^{-3}M, pH=7.40, T=37C, KCl=7.00 x 10^{-3}M, volume 230 ml., Titrant=$CaCl_2$= 8.00 x 10^{-3}M; KH_2PO_4=4.80 x 10^{-3}M + KOH=9.91 x 10^{-3}M.

($H_6L^{b}=H_5L^{-}+H^{+}$, pK=0.68; $H_5L^{5-}=H_4L^{2-}+H^{+}$, pK=2.21; $H_4L^{2-}=H_3L^{3-}+H^{+}$, pK=3.52; $H_3L^{3-}=H_2L^{4-}+H^{+}$, pK=5.09; $H_2L^{4-}=HL^{5-}+H^{+}$, pK=6.32; $HL^{5-}=L^{6-}+H^{+}$, pK=7.49 (Ref. 32); $Ca^{2+}+L^{6-}=CaL^{4-}$, pK=3.48; $2Ca^{2+} + L^{6-}=Ca_2L^{2-}$, pK=2.56 (Ref. 33).

a=H_3PO_4 dissociation constants and calcium phosphate ion-pair association constant not included; see Ref. (31).

above equation for various calcium phosphate phases indicate the thermododynamic stability of the experimental solutions compared to solutions in thermodynamic equilibrium with that particular phase. Positive ΔG values represent solutions undersaturated and negative ΔG values represent solution supersaturated with respect to solid phase under consideration.

The initial conditions used in the crystal growth experiments of HAP in the presence of mellitic acid are summarized in Table I. The analytical data for a typical crystal growth experiment in the presence of mellitic acid ($4.0x10^{-7}$M) are shown in Table II. It can be seen that the stoichiometry of the precipitating phase was

TABLE II

CRYSTALLIZATION OF HAP ON HAP SEED CRYSTALS AT CONSTANT SUPERSATURATION, pH=7.40, T=37C, MELLITIC ACID=4.00×10^{-7}M, EXP 10

Time (min.)	T_{Ca} 10^{-3}M	T_P 10^{-3}M	Extent of Crystallization (as % of original seed)
0	0.500	0.300	0
60	0.503	0.302	6.3
90	0.498	0.293	11.5
130	0.495	0.302	16.1
170	0.508	0.301	21.1
220	0.502	0.305	30.2

constant with calcium/phosphate molar ratio of 1.66 $\pm$ .02 for more than 3 hours of reaction. The amount of newly grown HAP phase was more than 30% of the original seed material. Plots of moles HAP/m^2 grown as a function of time for crystal growth experiments in the presence of mellitic acid, after making correction of the raw data for the observed changes in specific surface area, are shown in Figure 1. The striking constant rate of crystallization shown in Figure 1 suggests the development of a constant number of active growth sites on HAP seed crystals.

The results summarized in Table III indicate that the striking inhibitory effect of MA at low concentrations on the rate of HAP crystal growth cannot be attributed simply to the change in supersaturation due to complex formation. The curvature observed in Figure 1 at higher concentration of MA may be due to slow equilibration of the adsorbate with the surface of HAP seed crystals.

If the inhibition of HAP crystallization by mellitic acid is due to surface adsorption at active growth sites, some form of adsorption isotherm should be applicable. In many instances the Langmuir adsorption model which was developed for the adsorption of ideal gases onto solid surfaces, has been used to describe, empirically, the reduction in crystal growth rates of many sparingly soluble salts for a variety of inhibitors.

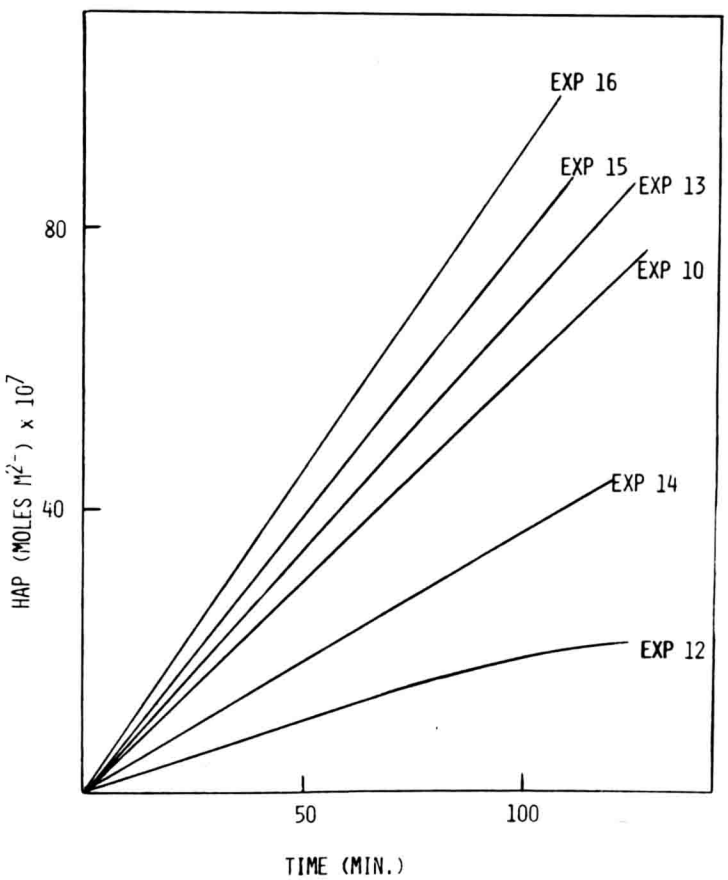

Figure 1: Crystal Growth of HAP on HAP Seed Crystals in the Presence of Mellitic Acid. Plots of HAP (moles m^{2-}) as a Function of Time.

TABLE III

CRYSTALLIZATION OF HAP ON HAP SEED CRYSTALS IN THE PRESENCE OF MELLITIC ACID

Exp	Mellitic Acid x 10^6 M	Rate x 10^8 (mole $min^{-1}m^{-2}$)	$\frac{R_o}{R_o-R_i}$
16	0.0	11.2	----
15	0.160	9.08	5.16
13	0.250	8.04	3.55
10	0.400	6.79	2.54
14	1.00	4.33	1.63
12	3.00	1.94	1.21

$T_{ca} = 0.500 \times 10^{-3}$ M; Tp = 0.300×10^{3} M; pH = 7.40; T = 37C

The familiar expression describing the Langmuir adsorption of solute is given by:

$$\theta = \frac{b\ [C]}{1+b\ [C]} \qquad (2)$$

where θ is the fraction of the surface covered by adsorbing solute (in this case mellitic acid), [C] is the solution concentration of the adsorbing substance, and b is the "affinity constant" for the solute-surface interaction. The rate of HAP crystallization in the presence of the mellitic acid, R_i, is proportional to the fraction of surface free from adsorbed meelitic acid, $(1-\theta)$, and is given by

$$R_i = (1-\theta)\ R_o \qquad (3)$$

where R_o is the HAP crystallization rate in the absence of mellitic acid. Equation (3) can be rearranged to give

$$\theta = \frac{R_o-R_i}{R_o} \qquad (4)$$

which on substitution in Equation (2) and upon further rearrangement, yield Equation (5)

$$\frac{R_o}{R_o - R_i} = \frac{1}{b[C]} + 1 \qquad (5)$$

According to Langmuir adsorption model, plot of R_o/R_o-R_i against $1/C$ should give a straight line. Plots of R_o/R_o-R_i against $1/C$ are shown in Figure 2 for the HAP crystal growth in the presence of MA. The excellent linearity suggests that the inhibitory effect of MA is due to adsorption at active growth sites. The value of "affinity constant" as calculated from Figure 2, is 1600×10^4 which can be compared with 98×10^4, and 24×10^4 for EHDP and pyrophosphate,[34] respectively. It is of interest to note that MA has also been found to exhibit greater inhibitory effect than EHDP on the crystal growth of fluorapatite as studied by constant composition method.[34]

Frances et al[21] studied the spontaneous precipitation of calcium phosphate (4.0mM $CaCl_2$, 4.0mM KH_2PO_4, pH 7.40, 37C) in the presence of MA and EHDP, and concluded that EHDP is a more effective crystal growth inhibitor than MA. This is in contrast with the results obtained in the present study where only HAP exclusively grew on the HAP seed crystals in the presence of MA, compared to the formation of calcium phosphate precursor phases under spontaneous precipitation.[21] Thus it is likely that mellitic acid may have a different affect on the formation and subsequent transformation of the precursor phase than on the formation of the thermodynamically stable phase. The inhibitory effect of mellitic acid on the crystallization of CaF_2 has also been recently investigated[35] using spontaneous and constant composition techniques. The results of this study indicate that mellitic acid has only a small effect on the crystal growth of CaF_2 at high supersaturation, whereas at low supersaturation it has a striking inhibitory effect. The importance of the influence of supersaturation on the performance of inhibitors is thus clearly demonstrated in these studies.[21,34,35] Whereas the use of spontaneous precipitation method fails to demonstrate the effectiveness of inhibitors at high supersaturation, the use of constant composition method not only yields highly reproducible results but also offers the opportunity to assess the performance of inhibitors under conditions of practical importance.

In the present work it has been shown that the presence of mellitic acid at low concentration can significantly inhibit the crystal growth of HAP from calcium phosphate solutions of low supersaturation. The marked inhibitory influence of mellitic acid on the crystallization of HAP may be explained by adsorption of inhibitor molecules at the active growth sites.

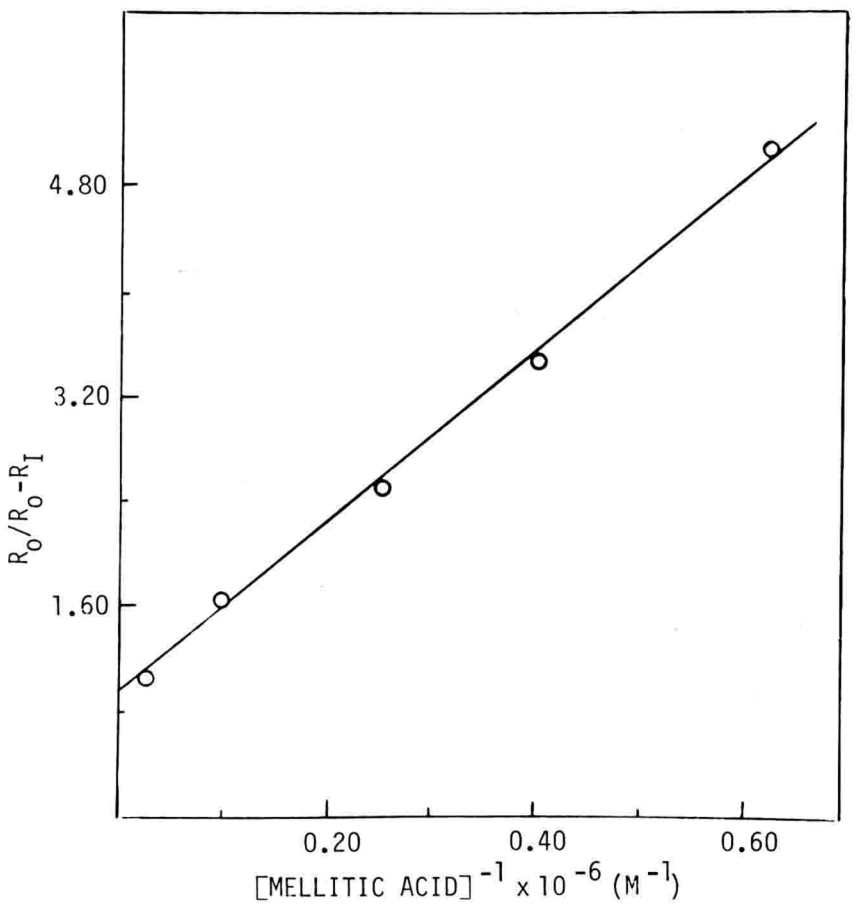

Figure 2: Crystal Growth of HAP on HAP Seed Crystals in the Presence of Mellitic Acid. Plots of R_o/R_o-R_i against 1/[Mellitic Acid].