NATIONAL BUREAU OF STANDARDS REPORT

10 155

Progress Report

on

PREPARATION OF HYDROXYAPATITE BY HYDROLYSIS OF α – TRICALCIUM PHOSPHATE

NBS

U.S. DEPARTMENT OF COMMERCE

NATIONAL BUREAU OF STANDARDS

NATIONAL BUREAU OF STANDARDS

The National Bureau of Standards[1] was established by an act of Congress March 3, 1901. Today, in addition to serving as the Nation's central measurement laboratory, the Bureau is a principal focal point in the Federal Government for assuring maximum application of the physical and engineering sciences to the advancement of technology in industry and commerce. To this end the Bureau conducts research and provides central national services in four broad program areas. These are: (1) basic measurements and standards, (2) materials measurements and standards, (3) technological measurements and standards, and (4) transfer of technology.

The Bureau comprises the Institute for Basic Standards, the Institute for Materials Research, the Institute for Applied Technology, the Center for Radiation Research, the Center for Computer Sciences and Technology, and the Office for Information Programs.

THE INSTITUTE FOR BASIC STANDARDS provides the central basis within the United States of a complete and consistent system of physical measurement; coordinates that system with measurement systems of other nations; and furnishes essential services leading to accurate and uniform physical measurements throughout the Nation's scientific community, industry, and commerce. The Institute consists of an Office of Measurement Services and the following technical divisions:

Applied Mathematics—Electricity—Metrology—Mechanics—Heat—Atomic and Molecular Physics—Radio Physics[2]—Radio Engineering[2]—Time and Frequency[2]—Astrophysics[2]—Cryogenics.[2]

THE INSTITUTE FOR MATERIALS RESEARCH conducts materials research leading to improved methods of measurement standards, and data on the properties of well-characterized materials needed by industry, commerce, educational institutions, and Government; develops, produces, and distributes standard reference materials; relates the physical and chemical properties of materials to their behavior and their interaction with their environments; and provides advisory and research services to other Government agencies. The Institute consists of an Office of Standard Reference Materials and the following divisions:

Analytical Chemistry—Polymers—Metallurgy—Inorganic Materials—Physical Chemistry.

THE INSTITUTE FOR APPLIED TECHNOLOGY provides technical services to promote the use of available technology and to facilitate technological innovation in industry and Government; cooperates with public and private organizations in the development of technological standards, and test methodologies; and provides advisory and research services for Federal, state, and local government agencies. The Institute consists of the following technical divisions and offices:

Engineering Standards—Weights and Measures — Invention and Innovation — Vehicle Systems Research—Product Evaluation—Building Research—Instrument Shops—Measurement Engineering—Electronic Technology—Technical Analysis.

THE CENTER FOR RADIATION RESEARCH engages in research, measurement, and application of radiation to the solution of Bureau mission problems and the problems of other agencies and institutions. The Center consists of the following divisions:

Reactor Radiation—Linac Radiation—Nuclear Radiation—Applied Radiation.

THE CENTER FOR COMPUTER SCIENCES AND TECHNOLOGY conducts research and provides technical services designed to aid Government agencies in the selection, acquisition, and effective use of automatic data processing equipment; and serves as the principal focus for the development of Federal standards for automatic data processing equipment, techniques, and computer languages. The Center consists of the following offices and divisions:

Information Processing Standards—Computer Information — Computer Services — Systems Development—Information Processing Technology.

THE OFFICE FOR INFORMATION PROGRAMS promotes optimum dissemination and accessibility of scientific information generated within NBS and other agencies of the Federal government; promotes the development of the National Standard Reference Data System and a system of information analysis centers dealing with the broader aspects of the National Measurement System, and provides appropriate services to ensure that the NBS staff has optimum accessibility to the scientific information of the world. The Office consists of the following organizational units:

Office of Standard Reference Data—Clearinghouse for Federal Scientific and Technical Information[3]—Office of Technical Information and Publications—Library—Office of Public Information—Office of International Relations.

---

[1] Headquarters and Laboratories at Gaithersburg, Maryland, unless otherwise noted; mailing address Washington, D.C. 20234.

[2] Located at Boulder, Colorado 80302.

[3] Located at 5285 Port Royal Road, Springfield, Virginia 22151.

NATIONAL BUREAU OF STANDARDS REPORT

NBS PROJECT
311.05-11-3110561

December 31, 1969

NBS REPORT
10 155

Progress Report
on

PREPARATION OF HYDROXYAPATITE BY HYDROLYSIS OF α – TRICALCIUM PHOSPHATE

by

Elmer T. Carlson* and Edgard C. Moreno**

* Research Associate from the American Dental Association at the National Bureau of Standards, Washington, D. C. 20234.

** Research Associate from the American Dental Association at the Dental Research Section, National Bureau of Standards, Washington, D. C., currently at the Forsyth Dental Center.

This investigation was supported in part by research grant DE02659 to the American Dental Association from the National Institute of Dental Research and is part of the dental research program conducted by the National Bureau of Standards in cooperation with the Council on Dental Research of the American Dental Association; the U. S. Army Medical Research and Development Command; the Dental Sciences Division of the School of Aerospace Medicine, USAF; the National Institute of Dental Research; and the Veterans Administration.

NBS

U.S. DEPARTMENT OF COMMERCE

NATIONAL BUREAU OF STANDARDS

Preparation of Hydroxyapatite by Hydrolysis of α - Tricalcium Phosphate

Elmer T. Carlson* and Edgard C. Moreno**

Institute for Materials Research
National Bureau of Standards
Washington, D. C. 20234

This investigation was supported in part by research grant DE02659 to the American Dental Association from the National Institute of Dental Research and is part of the dental research program conducted by the National Bureau of Standards, in cooperation with the Council on Dental Research of the American Dental Association; the United States Army Medical Research and Development Command; the Dental Sciences Division of the School of Aerospace Medicine, USAF; the National Institute of Dental Research; and the Veterans Administration.

* Research Associate from the American Dental Association at the National Bureau of Standards

** Research Associate from the American Dental Association at the National Bureau of Standards, currently at Forsyth Dental Center.

1. Introduction

Hydroxyapatite (HA), $Ca_5OH(PO_4)_3$, prepared in the laboratory by precipitation methods, is always an extremely fine-grained material, even after prolonged boiling. There have been numerous attempts at synthesis of HA crystals sufficiently large to be clearly resolved by the optical microscope, and to permit their crystal structure to be studied by single-crystal methods. W. Rathje, in 1941,[1] published a report of an "acidimetric precipitation" process whereby he prepared microscopically visible crystals of hydroxyapatite. Solutions of calcium nitrate and monobasic potassium phosphate were mixed dropwise in the correct stoichiometric ratio for hydroxyapatite, and the solution was kept neutral by simultaneous addition of sodium hydroxide solution. By working with very dilute solutions near the boiling point and adding the reagents at the rate of 5 drops per day, he succeeded in obtaining polycrystalline aggregates with individual crystals apparently 30 μm or more in length.

In the production of quantities of HA adequate for various experimental studies it has been necessary to sacrifice crystal size to increase the yield and to keep within a reasonable time schedule. For example, the preparation used by Moreno, Gregory and Brown in their solubility studies[2] was reported to contain "a minor fraction of thin needles up to 10μm in length," the rest consisting of smaller, irregular equant grains.

Hydrothermal methods have been more successful in the production of larger crystals of hydroxyapatite. The presence of sodium hydroxide appears to promote crystal growth. Thus Hayek and co-workers[3] prepared crystals up to 2 mm in length by hydrothermal treatment of a previously prepared fine-grained specimen of hydroxyapatite, in the presence of NaOH. It should be noted, however, that approximately one atom-percent of sodium was found by analysis to be present in the crystals. More recently, Mosebach[4] reported production of crystals up to 4 mm long by hydrothermal treatment of a mixture of KH_2PO_4, $Ca(NO_3)_2$ and NaOH for 10 days at 500°C and 360 atm. No analysis was reported.

Perloff and Posner[5] synthesized crystals of hydroxyapatite up to 0.3 mm in length by hydrolysis of dicalcium phosphate under hydrothermal conditions, thus avoiding the presence of extraneous ions. With the equipment available to them, the quantity obtainable in one batch was limited to about 0.1 g. It is clear that a limiting factor here is the increase in acidity of the solution as the acid phosphate dissolves and the basic hydroxyapatite is precipitated. Recent attempts in this laboratory to repeat the synthesis by the method of Perloff and Posner produced crystals only slightly longer than 0.1 mm. Recrystallized dicalcium phosphate was also present.

The fact that the methods used for preparing hydroxyapatite crystals of desired size and purity for single-crystal work have yielded only minute quantities points to the need for additional crystallization effort.

Although the processes of nucleation and growth of hydroxyapatite crystals are not understood, it seems evident that, at the boiling temperature and below, the rate of growth on the surface of crystals already present is slow, relative to the rate of formation of new crystals. Attention has, therefore, been given to methods of controlling the degree of supersaturation, in the expectation that a very low degree of supersaturation might inhibit nucleation while still permitting growth on crystal faces. One obvious method of increasing the concentration slowly is to start with a solid substance which dissolves slowly, yielding calcium and phosphate ions, and preferably no others aside from H^+ or OH^-. Hydroxyapatite itself, in poorly crystalline form, would appear to be a logical starting material, but experience has shown that only a slight degree of crystal growth is achieved even after prolonged digestion at the boiling temperature.

Dicalcium phosphate is another possible source of Ca^{++} and $PO_4^{\equiv}$ ions, and many attempts have been made to prepare hydroxyapatite crystals by hydrolysis of this compound or its hydrate, with appropriate control of pH. However, it dissolves rather rapidly, thus affording little advantage over precipitation methods.

Tricalcium phosphate (TCP) is much less readily soluble than $CaHPO_4$, and for this reason it seemed that it might serve as a suitable source of Ca^{++} and $PO_4^{\equiv}$ ions. Tricalcium phosphate occurs in three crystalline forms. The most common form, β-$Ca_3(PO_4)_2$ or whitlockite, is stable up to about 1100°C. At higher temperatures the αform is stable, but it can also exist at room temperature if it has been cooled rapidly enough. A third modification apparently exists only above 1430°C[6], and hence need not be of concern here.

The scientific literature relative to TCP is contradictory in some respects. For example, temperatures reported by different authors for the α-β inversion temperature span several hundred degrees. Nurse, Welch, and Gutt[6] placed this inversion point at 1125°C on the basis of heating curves, but stated that the curve dropped steeply in the presence of pyrophosphate. The latter phase, of course, would be expected only if the composition departed from TCP stoichiometry in the acid direction. In a later paper[7], Welch and Gutt did not mention the lowering of the inversion temperature in this range; on the other hand, they reported finding β-TCP in samples quenched from above 1125°C. It appears that the inversion may be sluggish in both directions, which would explain, at least in part, the uncertainty regarding the inversion temperature. From a practical standpoint, whatever the reason, the preparation of α-TCP free from the β-phase is difficult.

There appears to have been no systematic study of the effects of heat treatment and stoichiometry on the properties of the product, including solubility.

2. Experimental Procedures

2.1 Preparation of Tricalcium Phosphate

Several lots of β-TCP were on hand from an earlier study. Three of these (designated lots 1, 2 and 3) were reheated at a higher temperature to prepare the α-TCP needed for the present work. Additional batches of α-TCP (lots 4, 5 and 6) were later prepared directly from the raw materials. Because of differences in behavior among the various preparations, it is appropriate to describe the methods in some detail.

With one exception, all the lots of β-TCP were made from mixtures of dicalcium phosphate ($CaHPO_4$) and calcium carbonate. The exception was made by adding calcium carbonate to a solution of monocalcium phosphate ($CaH_4(PO_4)_2H_2O$) in water. After filtration, drying, and heating, it was treated like the others. This preparation was not used in making the α-phase; however, it will be referred to later.

The $CaHPO_4$ and $CaCO_3$ were mixed in various ways, but all involved suspension in alcohol. The methods included wet sieving, grinding a thick suspension in a mortar, shaking in a flask with Teflon beads, and stirring in a Waring Blendor. The last two methods in particular might have been expected to effect very thorough mixing, yet evidence of non-uniformity was found in all cases. It is assumed that segregation occurred during filtration and drying. The dried mixture was then heated for a day or longer, usually between 950 and 1100°C. The cooled product was examined microscopically and analyzed. In all cases some adjustment of composition was needed, and the process was repeated after appropriate addition of phosphate or $CaCO_3$, or, in some cases, $Ca(OH)_2$ in solution. Even after the composition was satisfactory and after reheating for several days, inhomogeneities were noted microscopically. The batches were therefore heated to 1150-1200°C, converting the TCP to the α-form, and then reconverted to the β-form by prolonged heating at 800-950°C.

Microscopically, the β-TCP preparations consisted of aggregates of rounded grains ranging from 7 to 50μm in diameter. Except for some variation in average particle size, the different lots (including that prepared from monocalcium phosphate) appeared identical. The Ca/P ratio (molar) was 1.50, within the limits of analytical error, and the percentage of phosphorus present as pyrophosphate was 0.30 or less, which is within the uncertainty of the analytical method. X-ray powder patterns showed β-TCP, with a few barely detectable peaks that could be attributed to α-TCP.

As mentioned above, ∝-TCP was prepared from the β-form by heat treatment at 1150-1200°C. Microscopic examination revealed the presence of some β-TCP in all such preparations, ranging from less than 1% to as much as 10%. It was not always clear whether this was residual or whether it had formed during cooling, but in many cases the particle size and the distribution indicated the latter case. For unexplained reasons, the proportion of the β-phase was often much higher in the layer in contact with the platinum container than in the bulk of the sintered mass. Efforts to reduce the amount of β-TCP by speeding up the cooling were not consistently successful.

For the direct preparation of ∝-TCP the method of mixing the raw materials in alcohol was abandoned in favor of dry mixing. The procedure ultimately adopted as standard was to mix the dry ingredients briefly by shaking, then to sieve 3 times through a No. 200 (74 μm) stainless steel sieve, and to heat the well compacted batch at 1200°C for 24 hours or longer. After cooling in air, the material was ground to pass the No. 200 sieve, examined, analyzed, and given such further heat treatment as was desired. Some β-TCP was observed in these preparations also.

Further details as to heating and cooling treatment will be given below, whenever pertinent.

2.2 Hydrolysis Procedures

The general procedure followed was very simple. A weighed quantity of the pulverized $Ca_3(PO_4)_2$ was added to a measured quantity of distilled water, (usually 100 or 200ml) which had been previously boiled to expel CO_2, in a 300-ml Erlenmeyer flask. A few drops of bromthymol blue were added to serve as an approximate measure of pH. The flask was fitted with a rubber stopper through which a glass tube about 20 inches long extended, serving as an air condenser. The contents were heated quickly to boiling and the flask placed on a hotplate, where boiling was continued as long as warranted by the results. Progress of the reaction was checked occasionally by microscopic examination of a drop of the suspension. Minor modifications of the procedure were introduced from time to time; these are indicated in the discussion of the results below.

Following a long series of experiments in flasks, operations were continued on a larger scale. The vessel was a stainless steel kettle, Teflon-lined, with a capacity of about 13 liters, fitted with a lid of the same material which could be clamped on. There were several holes in the lid, fitted with Teflon bushings drilled to accept standard taper glass joints. Contents were stirred by a motor-driven propeller, 9 inches from tip to tip, moving at about 160 r.p.m. The propeller and shaft were gold plated. A glass condenser was fitted on top, and a slow current of nitrogen was bubbled through the suspension. The kettle was heated by a hotplate.

3. Experimental Results

3.1 Preliminary

One-gram samples of α-TCP and β-TCP were placed in bottles with 100 ml distilled water and kept for several months. No hydrolysis was apparent. Similar samples were placed in flasks with 200 ml water and boiled. The β-TCP was unaltered after 18 hours boiling, but the α-TCP was appreciably changed in a few hours. The greater part of the subsequent experimental work has been devoted to α-TCP at boiling temperature.

3.2 Hydrolysis of α - Tricalcium Phosphate

The sequence of changes observed during the hydrolysis of α-TCP is the same, except for rate and degree of completion, for most of the experiments described below, and will therefore be discussed in some detail. When α-TCP (prepared as described above) is added to hot water in the ratio of, for example, 0.2 g to 100 ml, the pH rises rapidly. If the solution contains bromthymol blue, an indicator which changes from yellow to blue over the pH range 6.0-7.6, the change is observed as a deepening blue. The liquid remains clear, and at first there is no change in the microscopic appearance of the TCP. From these observations it is inferred that the first reaction is a direct dissolution of TCP. After a few minutes or hours, depending on the solid-liquid ratio and other factors, the color slowly fades and eventually becomes yellow. At the same time there is a tendency of the TCP grains to agglomerate and to stick to the flask. Under the microscope the TCP grains are seen to be more or less covered with an overgrowth of rods, prisms, or needles radiating outward. This type of crystal growth is sometimes referred to as "drusy". In the early stages of the reaction, certain crystals or polycrystalline particles of TCP will be found entirely coated with the new growth, while other grains will be completely clean. In exceptional cases, particles have been observed in an incipient stage of alteration, with the new growth appearing as a well defined patch on an otherwise smooth surface. In other cases, formation of the rod-shaped crystals seems to occur more quickly at surface irregularities.

Under favorable conditions, the reaction will progress to the interior of the particles until no TCP remains. However, the reaction product in the interior apparently is unable to develop well formed crystals, and appears as irregular fragments after the aggregates are crushed. In several instances it was possible to examine fully hydrolyzed grains without crushing and several of these showed fairly well defined cores of material with refractive index lower than that of the radiating HA crystals. The cores were isotropic, showing that they were not simply unaltered α-TCP.

Figure 1 is a photomicrograph of a few dispersed crystals broken from the drusy aggregates. Several of the typical acicular crystals ("needles") are visible, as well as some of the smaller irregular fragments and uncrushed aggregates. Typically, the needles are 30 μm or less in length and seldom over 2 μm in diameter, too small to permit any prism faces to be discerned. It is possible, by careful manipulation of the cover glass, to rotate the crystals on the microscope slide, whereby it can be seen that they are rod-shaped rather than flat. Individual, slender needles up to 100 μm long have been observed, and occasionally there are thicker crystals, much shorter but as much as 5 μm in diameter. A few of these have been observed microscopically, oriented parallel to the microscope axis, and some of them appear hexagonal in shape. The indices of refraction of the needles, with unfiltered light, range from 1.630 to 1.632 and from 1.632 to 1.635 (±0.003) for the low and high indices, respectively. These figures are appreciably lower than the accepted values (1.640 and 1.647) for mineral hydroxyapatite, but are close to the average index for typical precipitated HA. Most of the irregularly shaped fragments show a somewhat lower refractive index, difficult to determine precisely because of the small particle size, but apparently close to 1.62. Minute particles with index appreciably below 1.62 are also observed in some of the preparations.

XRD patterns have been obtained for several of the hydrolysis products. In each case a strong pattern, characteristic of HA, was obtained. In general, extraneous peaks were either absent or so small as to be barely distinguishable from the background. In one pattern, however, two peaks of appreciable size were observed, corresponding in position to two of the strongest lines of β-TCP. The stronger was about 1/7 the height of the strongest HA peak, giving a rough indication of the relative amounts present. It should be noted here that the rest of the strong β-TCP lines are in positions where they are masked by nearly coincident HA lines.

The pattern of one of the other hydrolysis products showed the same two β-TCP lines, though in a considerably lower intensity. It is considered significant that both of these preparations showed unusually high amounts of the phase having an index of 1.62. On the basis of this evidence the phase is tentatively identified as β-TCP.

No other constituents have been even tentatively identified in the hydrolysis products, though there is indirect evidence (presented later) that one or more of the acid phosphates may be present.

Chemical analyses of the reaction products have been made only in relatively few cases, but the results are fairly consistent in showing a Ca/P ratio well below the theoretical value for HA (1.67). The average found is about 1.57. The discrepancy can be explained, at least in part, if it is assumed that other phosphates are formed along with HA, possibly when the hydrolysis has progressed to the interior of the TCP grains. This can perhaps be shown more clearly by consideration of a hypothetical initial reaction $5Ca_3(PO_4)_2 + 3H_2O = 3Ca_5OH(PO_4)_3 + H_3PO_4$. The formation of H_3PO_4 would result in a gradual lowering of pH, in accordance with observations.

The increasing acidity might reach a point at which β-TCP or an acid phosphate could precipitate, with the result that the overall composition of the residue would be more acid than that of HA.

The results of the preliminary experiments were sufficiently promising to warrant further study under more closely controlled conditions. The effects of several variables were investigated, and these will now be considered in some detail.

3.2.1 Variation Among Different Lots of α-TCP

It will be useful first to consider the variability in behavior among the several lots of α-TCP used, and the reproducibility of results with a given preparation. Since the primary aim of the study was to produce large crystals of HA, the size of the crystals and the rate at which they are formed are the prime considerations. As an approximate measure of the latter, the time required for the reaction to proceed to the point where the pH had fallen to 6, as shown by the indicator, was recorded. This, of course, is not a point that can be determined with precision, but it is adequate for comparative purposes. No attempt was made to determine the average crystal size of the product; instead, a search was made for the largest HA needle on the microscope slide, and this was taken as a measure of relative particle size. This method also is lacking in precision, but it has the advantage of being rapid.

In Table 1 are given the results of hydrolysis experiments with several different preparations of α-TCP at the same solid-liquid ratio (expressed as grams of solid per 100 ml of water) and with other conditions held constant. Experiments 1-5A and 1-5B are duplicates, done concurrently, to obtain an idea of the reproducibility of results. The same is true of 1-6A and 1-6B and 1-7A and 1-7B. There was a slight difference in reaction rate between 1-7A and 1-7B, as measured by the time required for the pH to drop to 6, whereas in the other two pairs there was no observable difference in rate. Slight differences in crystal size were noted for all three pairs of duplicates.

Among the different TCP preparations there is a fairly large spread in the time required to reach pH 6, ranging from 1½ to 6½ hours.

In column 3 of Table 1, is given the "age" of the TCP at the time of the hydrolysis experiment; that is, the elapsed time since the final heat treatment of the preparation. The data in Table 1 were selected for comparison because all the TCP preparations included were several months old at the time of hydrolysis. Evidence at hand indicated that aging might affect the behavior of the TCP. Some additional experiments were run as a check on this effect, and the data are presented in Table 2. There appears to be a trend toward decreased reactivity with aging, especially for periods beyond 4 months.

Because of the variability in behavior among the various lots of α-TCP it appeared desirable to make a brief study of the effects of minor variations in procedure during preparation of the TCP on its behavior in contact with water.

The three most obvious variables to consider are temperature, duration of heating, and rate of cooling. With the furnaces readily available, the upper limit of temperature was about 1230°C while the lower limit was fixed at about 1125°C because of formation of the β-phase at lower temperatures.

Within this limited range, no consistent relation was found between either temperature or duration of heating and the manner in which the product reacted with water. (This applies to all the α-TCP preparations referred to herein except lots 4b and 4c.)

Subsequently, the use of a high-temperature furnace in another division of the National Bureau of Standards was obtained. It was then possible to study the variation in behavior of a preparation of α-TCP that had been heated over a wider range of temperature. The data are given in Table 3. It may be seen that the TCP heated at 1200°C (lot 4a) is slightly more reactive than that heated at 1300°C and much more reactive than that heated at 1395°C.

The rate of cooling conceivably could control both the amount of the β-phase present and the amount of strain remaining in the crystals, either of which would be expected to affect the rate of solution. However, it is difficult to achieve very rapid cooling of quantities of the size needed in these studies. When the container was removed from the hot furnace and allowed to cool in air, the contents glowed visibly for one to four minutes, depending on the quantity of material. (Duration of glow is of some significance, since it coincides roughly with the temperature range in which the α-β inversion proceeds most readily.) Setting the containers in water had little effect. Some attempts were made to quench the hot α-TCP by immersing it or spraying it with water. The products obtained after drying were very rapid reacting, and the hydrolysis products greatly inferior. Further efforts along this line were abandoned. Minor differences in rate of cooling were effected by using smaller quantitites of TCP, and by spreading it in a thin layer in a large dish prior to heating. The results of hydrolysis experiments on these variously cooled lots of TCP were conflicting, so that no conclusions as to the effect of cooling rate can be offered.

With the possible exception of Lot 4c, heated at 1395°C, all the α-TCP preparations contained some β-TCP, the amount usually ranging up to 10 percent. Within this range, no correlation was apparent between the amount of the β-phase and the reactivity. In some specially prepared batches with much higher β-TCP content, the hydrolysis was definitely retarded.

3.2.2 Effect of Fineness

The fineness, or more correctly the surface area, of a crystalline solid affects its rate of dissolution. In the present case there are, strictly speaking, two effects to consider: the size of the grains and the formation of fresh broken surfaces by grinding. In practice, these were inseparable. The α-TCP prepared at 1200°C or lower is a soft sinter, readily crushed to polycrystalline grains that will pass a No. 100 sieve. The material prepared at 1395°C required vigorous grinding to reduce it to pass the same sieve, and the resultant grains were usually fragments of larger crystals.

Hydrolysis data are given in Table 4 for three lots of α-TCP, each separated into three fractions by dry sieving. The particle size ranges given are the nominal openings of the three standard sieves used, Nos. 100, 200, and 325. Lot No. 3a, typical of the α-TCP prepared by heating at 1200°C, was relatively unaffected by sieving, all three fractions reacting at about the same rate. On the other hand, Lot 4c (heated at 1395°C) shows extreme differences between the three fineness fractions. Lot 4b (1300°C) is intermediate in behavior, the two coarse fractions being about equal in reactivity while the finest fraction is appreciably quicker in its reaction. The difference is clearly related to the actual crystal size, which is not always the same as the gross particle size. In the TCP prepared at 1200°C, the larger grains consist of relatively loosely bound aggregates of smaller crystals, most of which are smaller than 30 μm. The aggregate grains are sometimes chainlike structures, sometimes rather porous clusters. Crushing these to <44 μm has relatively little effect on the surface area. In specimen 4b the actual crystals range up to 60 μm or more in size, so that crushing to <44 μm results in an increase in surface area. In the material prepared at 1395°C the crystal size was much larger and the porosity very low, so that even coarse grinding resulted in formation of new surfaces.

Experiments with other lots of α-TCP prepared at 1200°C are consistent with those shown for Lot 3a in Table 4. In general, the quality of the reaction product, from the standpoint of crystal size, was inferior for the finest size fractions.

3.2.3 Solid - Liquid Ratio

In the foregoing we have considered variability introduced during preparation and subsequent treatment of the ∝-TCP. Variations in the hydrolysis procedure will now be considered.

The rate of solution of a solid in a given volume of liquid is related to the surface area in contact with the liquid which varies directly as the mass of the solid (assuming uniform size distribution). Thus the solid-liquid ratio, expressed here as grams of solid per 100 ml of water, was expected to control the rate of solution, and this presumably would affect the rate of the overall hydrolysis process. This proved to be the case. Table 5 gives the pertinent data for a series of hydrolysis experiments on a single lot of ∝-TCP, designated Lot 3a. The time required to reach pH6 increases as the solid-liquid ratio decreases. The relative reaction rate (reciprocal of time) is, very roughly, proportional to the solid-liquid ratio. Crystal size in the product increases as the solid-liquid ratio decreases. In the low-ratio suspensions, the hydrolysis was still incomplete after the indicated periods. Those with greater amounts of the solid present were essentially completely hydrolyzed, but contained larger amounts of low-index material in relation to the HA needles present.

The molar ratio, Ca/P in the hydrolysis product was determined by chemical analysis for several of the experiments in this series. There is a significant increase in this ratio as the solid-liquid ratio is lowered, approaching the theoretical value for HA (1.667). Along with this there is a decrease in the relative amount of the contaminating phase or phases ("low-index material") at the lower solid-liquid ratios.

For experiments 5-7 and 5-8 a larger flask was used, in order to have enough solid material for examination and analysis. The flask had standard-taper glass fittings, thus avoiding the possible undersirable effect of using rubber stoppers for the relatively long reaction period required. Experiment 5-8 was an attempt to determine whether the solid-liquid ratio could be reduced to a practical minimum without inhibiting the hydrolysis completely. This was shown to be the case. The reaction proceeded in the normal fashion but at a slower rate.

Comparable data were obtained for the other lots of ∝-TCP, though over a less extended range. Within a given batch, the same dependence of reaction rate on solid-liquid ratio was observed.

The IR spectra for several samples having a Ca/P ratio in the range 1.56 to 1.59 were consistent with the presence of acid phosphate groups in the samples. Likewise, after pyrolysis of these samples at around 460°C, the IR spectra showed peaks corresponding to the presence of pyrophosphate groups, as could have been anticipated.[8]

In further reference to Table 5, it was expected that the Ca/P ratio found in experiment 5-8 might be higher than in 5-6, but the results proved to be identical. The filtrate from 5-8 was evaporated to dryness and the nonvolatile residue was weighed. The weight of the residue was found to be considerably greater than that of the TCP initially present, and qualitative tests indicated the presence of silica. It was concluded that the flask had been attacked by the solution, which is not surprising when it is recalled that the pH was on the alkaline side during much of the 14-day period of boiling. It is not known whether the solid hydrolysis product also was contaminated with SiO_2, but this is considered a possibility. The presence of SiO_2 would interfere with the analytical determination of phosphorus, resulting in a high value which in turn would make the calculated Ca/P ratio too low.

It was decided to repeat one of the experiments in Table 5, making use of the Teflon-lined steel vessel described in Section 2.2. For practical reasons, the solid-liquid ratio selected was that of experiment 5-6 rather than 5-8. The volume of water used was 4 liters. After 4 days boiling, hydrolysis appeared to be complete, and analysis indicated a Ca/P ratio of 1.67, the theoretical value for HA. This is to be compared with the value 1.63 obtained for the same materials in the same ratio (but smaller volume) in glass (experiment 5-6).

3.2.4 Effect of Temperature During Hydrolysis

The hydrolysis experiments described in the preceding sections were carried out at boiling temperature. Table 6 gives some data on a series of similar experiments at lower temperatures. The rate of the reaction falls off at lower temperatures, as would be expected. The appearance of the hydrolysis product also changes, though between 90 and 100°C there is no great difference. Below 90°C the needles become smaller and the amount of irregularly shaped grains proportionately greater. Even at 25°C there was evidence of hydrolysis, though the reaction was incomplete during the period indicated.

3.2.5 Effect of Agitation

As mentioned earlier, one of the phenomena observed during the hydrolysis is a tendency of the solid residue to stick together and to the bottom of the flask. At first, attempts were made to prevent this by frequent shaking of the flask. Later a hotplate with magnetic stirrer was used. It was soon observed, however, that the frequent or continuous agitation usually slowed the hydrolysis reaction and invariably resulted in smaller HA crystals. The reasons for this are not clear, but for practical reasons the practice of agitating the suspension was abandoned. Even vigorous boiling was found to be detrimental, better crystals being obtained with gentle boiling or even two or three degrees below the boiling point. It is possible that the formation of larger crystals is related in some way to localized concentration gradients that may be disturbed by agitation of the solution.

3.2.6 Effects of Addition of $Ca(OH)_2$

In some of the preliminary experiments, $Ca(OH)_2$ was added to the reaction mixture to bring the stoichiometry up to that of HA. This was found to inhibit the reaction completely. The next step was to start with α-TCP in water and add $Ca(OH)_2$ dropwise to the boiling mixture to keep the pH at approximately 7. In this way, the conversion of TCP was apparently complete, but there was an amount of gelatinous or finely crystalline material, presumably a precipitate of calcium phosphate of undetermined composition.

After it had been proved by chemical analysis that the overall composition of the hydrolysis products was low in calcium, in comparison to HA, further attempts were made to correct the composition by adding $Ca(OH)_2$ to the solution. Coarsely crystalline $Ca(OH)_2$, prepared by slowly heating a saturated solution to boiling, was added to the reaction mixture, either at the start or in increments after the reaction commenced. It was hoped that the rate of dissolution of the $Ca(OH)_2$ would be slow enough to prevent precipitation of an amorphous or fine-grained phosphate. When added at the start, the $Ca(OH)_2$ dissolved too quickly, and inhibited the hydrolysis reaction. When added during the hydrolysis, it served to keep the solution neutral; nevertheless, the hydrolysis product, on analysis, proved to have the Ca/P ratio 1.57, approximately the same as the average found in the absence of added $Ca(OH)_2$ for suspensions of the same solid-liquid ratio.

3.2.7 Effects of other Alkalizing Agents

In one series of experiments, NH_4OH was added to the suspension during boiling, to keep the solution neutral or alkaline. Again the Ca/P ratio in the residue came out at 1.57.

In another series, urea was substituted for NH_4OH. Urea is hydrolyzed more rapidly in acidic than in neutral solution, and the overall effect is to maintain a moderate degree of alkalinity. At concentrations up to about 0.25% there was a small improvement in the Ca/P ratio, but this effect was sharply reversed when the amount of urea was doubled. The matter was not pursued further.

Sodium hydroxide was also tried on one occasion. The product appeared unsatisfactory, and no analysis was made.

The addition of calcium carbonate, in amount sufficient to over come the deficiency in calcium, inhibited the hydrolysis, though not completely. After prolonged boiling, only a few needle crystals were observed.

Tetracalcium phosphate, added to α-TCP in amount sufficient to give the overall composition of HA, accelerated the hydrolysis, but the product was distinctly inferior.

3.2.8 Effects of the Presence of Other Substances

In a few of the earliest experiments the water was not boiled to expel CO_2 before use. There was no apparent difference in the reaction or in the product after the practice of boiling out the CO_2 was adopted.

In one experiment, calcium pyrophosphate was added in the amount of 1.5% of the weight of the TCP used (this being in the range of the amount that might be present as a contaminant in the TCP). The course of the reaction was unaffected, and the pyrophosphate was observed microscopically in the product, apparently unaltered.

3.2.9 Production in Larger Quantity

Following the determination of the conditions favoring good crystal formation on the basis of experience gained in the work described above, two larger batches of HA were prepared in the stainless steel kettle described in the section on Procedures. For the first, 25 grams of α-TCP, Lot 2a, was ground to pass a No. 200 sieve, added to 5 liters of water, and the mixture boiled 21 hours with constant stirring. The product was essentially all HA, but the crystals were disappointingly small--about 10 μm and smaller. Analysis indicated a Ca/P ratio of 1.56. The second batch used 29 grams of the same TCP in 9.9 liters of water. After 20 hours boiling it was filtered off, dried in air at about 70°C and examined. There was some residual TCP, so the product was returned to the kettle with part of the solution and boiling was resumed for 45 hours. After this treatment, the TCP had been reduced to less than 0.1%. Maximum crystal length observed was 35 μm. The Ca/P ratio was 1.60.

3.3 Attempts at Further Crystal Growth

When it became apparent that varying the experimental conditions was unlikely to produce HA crystals larger than 60 μm, or exceptionally 100 μm, in length, a number of attempts were made to enlarge the crystals already produced, by keeping them in contact with calcium phosphate solutions supersaturated with respect to HA. These were completely unsuccessful. At room temperature, nothing happened. At 45°C, rosettes of octacalcium phosphate slowly formed. At higher temperatures, there was a gelatinous precipitate.

In other experiments, the hydrolysis reaction was permitted to proceed to varying stages of completion, then the bulk of the residue was removed, leaving only a few crystals to serve as seeds for further precipitation. Subsequent evaporation to a small volume was also tried. These experiments were equally unsuccessful.

3.4 Attempts at Removal of Acid Phosphates

It was pointed out above that the Ca/P ratio found by analysis of the hydrolysis product is always lower than the theoretical ratio for HA, and that the product is contaminated with some material, presumably one or more other phosphates, having an average refractive index slightly lower than that of HA. Efforts were made to remove this material by extraction with water and with 10^{-3}M H_3PO_4. Two successive extractions of 1.5g of residue with 1 liter of boiling water, dissolved only 0.1% of the residue. Extraction with the acid removed over 11%, but the increase in Ca/P ratio was so slight as to be within the limits of precision of the analytical method.

An attempt was also made at increasing the Ca/P ratio by heating the residue with $Ca(OH)_2$ solution. Again the slight increase found was within the limits of analytical precision.

3.5 Reaction of other Phosphates with Water

3.5.1 β-TCP

As mentioned earlier, the initial experiments with β-TCP were almost completely negative, only a few needle crystals being observed after many hours boiling.

Later the experiments were repeated with all of the four lots of β-TCP available, plus one freshly prepared. Four of these yielded the same negative results, but one reacted very much as did some of the less reactive lots of α-TCP. For example, at a solid-liquid ratio of 1 gram/100 ml, the solid was largely but not completely converted to HA needles and irregular grains after 5 hours boiling. The only difference between this lot of β-TCP and the others is that it was prepared from monocalcium phosphate and calcium carbonate rather than dicalcium phosphate and $CaCO_3$. Subsequent heat treatment was essentially the same, and there was no difference in analysis or in microscopic appearance.

3.5.2 Other Calcium Phosphates

A few experiments were run with calcium phosphate preparations richer in calcium than TCP (up to HA composition). They probably consisted of TCP and HA in different proportions. These reacted rather rapidly with boiling water, but yielded only small needles and much irregular material.

Tetracalcium phosphate, prepared at 1500°C, produced an alkaline solution with boiling water, but apparently was not hydrolyzed after several hours boiling.

4. Discussion

Preparation of hydroxyapatite by hydrolysis of α-tricalcium phosphate in water at or near the boiling point appears to be a promising method if moderate quantities of small but clearly defined crystals are desired. It has the advantage of avoiding the presence of foreign ions, and the process can be completed in a day, or in a few days at most.

Unfortunately, the method also has certain limitations, at least in the light of present information. Most serious of these is contamination with other phases. Part of this material has been identified as β-TCP on the basis of XRD and microscopic evidence. The presence of this phase in the hydrolysis products might be assumed to result from the β-TCP present as a contaminant in the α-TCP before hydrolysis. This may indeed be the origin of part of it, but there is some evidence that it is formed, at least in part, in the hydrolysis product. The material observed microscopically is often very finely divided and irregular in outline, whereas the original β-TCP grains are larger, nearly round, and well defined. Furthermore, the amount revealed by XRD in the case referred to earlier appears to be greater than that present in the α-TCP, whereas most of the other hydrolysis products for which XRD patterns were obtained show no β-TCP at all.

Acid phosphates have not been clearly identified in the hydrolysis products by either XRD or microscopy, but the formation of pyrophosphate on heating strongly suggests that an acid phosphate is present. This could be either octacalcium phosphate or dicalcium phosphate, or a mixture of the two. So far, no practical means of eliminating these contaminants has been found. Apparently they can be minimized, however, by decreasing the solid-liquid ratio to the lowest practical point. Under such conditions, the composition of the acicular crystals is close to the theoretical value of HA. Obviously, lowering the solid-liquid ratio has the serious disadvantage of giving a low yield.

The use of glass vessels should also be avoided, because the persistent alkalinity and the long reaction period accompanying the use of a low solid-liquid ratio are conducive to attack on the glass and possible contamination of the product.

There is need for further information on the crystallography of the product, but no crystals large enough for single-crystal x-ray study have been obtained so far. The relatively low refractive index of the crystals (averaging about 1.633) is closer to that of the familiar precipitated HA than to the natural or the hydrothermally prepared materials. The x-ray powder pattern is typical of HA, but this in itself is not a clear indication that the composition is strictly stoichiometric. The presence of other phosphates in small amounts would not necessarily be revealed by x-ray powder patterns.

Much remains to be learned about the mechanism of the precipitation of the HA crystals. Typically, the needles or rods project radially from the surface of the TCP grains, which generally are relatively much larger. Some nucleation sites are evidently more active than others, because a partially altered suspension of TCP will usually show some TCP grains heavily coated with HA while others appear unaltered. As long as the HA crystals can develop freely into the surrounding liquid they are well formed. As the reaction progresses into the interior of a large TCP grain, the development of HA presumably is impeded by the crust of crystals already formed, with the result that poorly formed or fragmented crystals are produced in the interior. Restricted diffusion of ions in this area may lead to coprecipitation of β-TCP or acid phosphates.

In summary, it has been shown that the hydrolysis of α-TCP at, or near, the boiling point will result in acicular crystals of HA ranging up to 100 μm in length by 1-2 μm in diameter, but that the problem of preventing or removing other coprecipitated phases, while still obtaining a reasonable yield, has not been solved.

Acknowledgments. Several members of the National Bureau of Standards staff and American Dental Association research associates cooperated in various phases of this study. Special thanks are due to H. E. Swanson for the XRD patterns; to D. H. Blackburn for heating specimens at high temperatures; to C. P. Mabie, Jr. for IR absorption analyses and photomicrographs; and to B. M. Wallace and J. M. Palcic Patel for chemical analyses.

References

1. Rathje, W., Ber. d. deut. chem. Gesell. 74, 342-349 (1941).

2. Moreno, E. C., Gregory, T. M., and Brown, W. E., NBS Report 9631, Oct. 25, 1967.

3. Hayek, E., Böhler, W., Lechleitner, J., and Petter, H., Z. anorg. Chem. 295, 241-246 (1958).

4. Mosebach, R., Bull. Group. Int. Rech. Sc. Stomat. 9, 45-49 (1966).

5. Perloff, A., and Posner, A. S., Science 124, No. 3222, 583-584 (1956).

6. Nurse, R. W., Welch, J. H., and Gutt, W., J. Chem. Soc. (London) 1959, 1077-83.

7. Welch, J. H. and Gutt, W., J. Chem. Soc. (London) 1961, 4442-4444.

8. Fowler, B. O., Moreno, E. C., and Brown, W. E., Arch. oral Biol. 11, 477-492 (1966).

Table 1

Hydrolysis of α-TCP in boiling water; variation among several lots of α-TCP

Experiment No.	TCP Lot No.	Age of TCP	Solid-Liquid Ratio g/100 ml	Time taken to reach pH 6 hours	Max. crystal length μm
1-1	1 c	7 mo	0.20	$6\frac{1}{2}$	40
1-2	2 b	6 mo	0.20	$4\frac{1}{2}$	40
1-3	3 a	4 mo	0.20	2	35
1-4	3 b	4 mo	0.20	4	40
1-5A	4 a	6 mo	0.20	3	18
1-5B	4 a	6 mo	0.20	3	22
1-6A	4 b	6 mo	0.20	3	15
1-6B	4 b	6 mo	0.20	3	20
1-7A	5	4 mo	0.20	$1\frac{1}{2}$	35
1-7B	5	4 mo	0.20	$1\frac{2}{3}$	40

Table 2

Hydrolysis of α-TCP in boiling water after aging for various periods

Experiment No.	TCP Lot No.	Age of TCP	Solid-Liquid Ratio g/100 ml	Time taken to reach pH 6 hours	Max. crystal length µm
2-1	3 a	1 day	0.20	1½	30
2-2	3 a	4 mo	0.20	2	35
2-3	3 a	5 mo	0.20	3	30
2-4	3 a	6 mo	0.20	4	45
2-5	3 b	1 day	0.20	2	50
2-6	3 b	4 mo	0.20	4	40
2-7	4 a	1 day	0.20	1¼	25
2-8	4 a	6 mo	0.20	3	18
2-9	4 b	1 day	0.20	2	30
2-10	4 b	6 mo	0.20	3	20
2-11	5	1 day	0.15	1¾	30
2-12	5	4 mo	0.15	1½	35
2-13	6	1 day	0.15	2	25
2-14	6	4 mo	0.15	2¼	20

Table 3

Hydrolysis of α-TCP in boiling water; effect of temperature of preparation of α-TCP on rate of hydrolysis

Experiment No.	TCP Lot No.	Max. Temp. TCP heated °C	Solid-Liquid Ratio g/100 ml	Time taken to reach pH 6
3-1	4 a	1200	0.20	$1\frac{1}{4}$ hr.
3-2	4 b	1300	0.20	2 hr.
3-3	4 c	1395	0.20	9 days*

*pH still above 7 when run was terminated.

Table 4

Hydrolysis of α-TCP in boiling water; effect of fineness

Experiment No.	TCP Lot No.	Max. Temp. TCP heated °C	Particle size range μm*	Solid-liquid ratio g/100 ml	Time taken to reach pH 6	Max. crystal length μm
4-1	3 a	1200	74-149	0.20	2 hr.	30
4-2	3 a	1200	44-74	0.20	1½ hr.	35
4-3	3 a	1200	<44	0.20	1½ hr.	30
4-4	4 b	1300	74-149	0.10	10 hr.	30
4-5	4 b	1300	44-74	0.10	12 hr.	30
4-6	4 b	1300	<44	0.10	3 hr.	40
4-7	4 c	1395	74-149	0.20	>3 days**	18
4-8	4 c	1395	44-74	0.20	10 hr.	20
4-9	4 c	1395	<44	0.20	50 min.	12

* Nominal sieve opening

** pH above 7 when experiment was terminated after 3 days. Hydrolysis incomplete.

Table 5

Hydrolysis of α-TCP in boiling water;
effect of varying solid-liquid ratio

Experiment No.	TCP Lot No.	Solid-liquid ratio g/100 ml	Time taken to reach pH 6	Total Time	Ca/P (Molar) in Residue	Max. crystal length µm	Other substances in residue
5-1	3 a	0.80	50 min.	4 hr.	1.52	20	Much low-index material; XRD shows β-TCP. No α-TCP observed
5-2	3 a	.40	2 hr.	21 hr.	---	40	Much low-index material; no α-TCP observed
5-3	3 a	.20	3 hr.	24 hr.	---	50	Same
5-4	3 a	.10	4½ hr.	24 hr.	1.57	50	Little low-index material; no α-TCP observed
5-5	3 a	.075	18 hr.	24 hr.	---		
5-6	3 a	.05	30 hr.	48 hr.	1.63	70	Little low-index material; no α-TCP observed
5-7	3 a	.025	50 hr.	74 hr.	---	90	Very little low-index material; few crystals of α-TCP
5-8	3 a	.0020	**	14 days	1.63	75	Few minute grains with low-index few crystals of α-TCP

*Experiments 5-7 and 5-8 were run in a round bottomed flask, with heating mantle, fitted with a water condenser through glass joints. Volume of water 1500 ml.

**pH still above 6 after 14 days.

Table 6

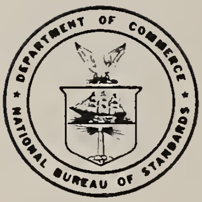
Hydrolysis of α-TCP in water;
effect of temperature during hydrolysis

Experiment No.	TCP Lot No.	Solid-liquid ratio g/100 ml	Temperature °C(±2)	Time taken to reach pH 6	Total time	Max. crystal length μm	Residue appearance
6-1	5	0.20	Boiling	$1\frac{1}{4}$ hr.	6 hr.	30	Mostly needles, some irregular grains
6-2	5	0.20	91	2 hr.	$5\frac{1}{2}$ hr.	25	Similar to 6-1
6-3	5	0.20	82	3 hr.	$6\frac{1}{2}$ hr.	25	Mostly irregular grains; some clusters of needles
6-4	5	0.20	72	$>6\frac{1}{2}$ hr.	$6\frac{1}{2}$ hr.	25	Mostly irregular grains
6-5	5	0.20	62	23 hr.	23 hr.	8	Mostly irregular grains
6-6	5	0.20	52	5 days	5 days	8	Mostly irregular grains
6-7	5	0.20	45	17 days	17 days	--	Incompletely hydrolyzed; fine irregular grains
6-8	5	0.20	Room temp.	60 days	77 days	--	Incompletely hydrolyzed; product "amorphous"

Figure 1.

HA crystals obtained on hydrolysis of α-TCP in boiling water.


10 µm


DEPARTMENT OF COMMERCE
NATIONAL BUREAU OF STANDARDS