Enhanced maturation of hydroxyapatite from aqueous solutions using microwave irradiation

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A new method for manufacturing a pure hydroxyapatite (HA) through precipitation from aqueous medium within less than an hour by using microwave irradiation is described and a physicochemical explanation is suggested.

Introduction

The microwave band, which is part of the electromagnetic spectrum, is usually defined as ranging between frequencies of 300 MHz and 300 GHz, i.e., wavelengths of 1 m to 1 mm. The major applications of the microwave energy are in the industrial heating or processing of wood, paper, food and textile products. These procedures employ the 3 MHz to 30 GHz range, with generated power of up to 70 kW. For pharamaceutical drying a specific radiation of 24 GHz is used [1].

Recently a new trend of using microwave energy for the enhancement of chemical reactions has evolved. Methods for causing chemical reactions in a solid phase, e.g., an enzyme which acts as a catalyst [2], have been reported.

Crystallization methods employing microwave radiation which can be especially useful in the manufacture of porous crystalline materials, e.g., zeolites [3] have been described. Crystal growth of diamond seeds in microwave-plasma chemical vapour deposition has been studied [4]. Microwaves are also employed for less spectacular substances: Microwave heating of a sucrose solution during preparation of its suspension, increased the crystal nucleation rate by a factor of > 2 and improved the uniformity of crystals in comparison with convective heating [5].

To the best of our knowledge most of these recent publications deal with preparative techniques. The mechanisms by which the absorption of electromagnetic energy in the microwave range affects a particular chemical reaction are not yet sufficiently clear to make predictions feasible.

Extensive studies have been carried out on the effects of microwave radiation on biological systems and especially on the human body. A specific aspect of these studies which may be of interest to the chemist, is the difference in mode of absorption of the microwave energy by bound water on the one hand, as opposed to free water on the other. The bound water consists of water of hydration on many of the cellular molecules, and of surface water weakly bonded to membranes and membrane surface molecular complexes. The bound water absorbs appreciably from 10 MHz to 10 GHz [6]. Grant *et al.* [7] presented, in graphic form, the relationship between the absorption of the energy in biological tissue due to free and bound water as a function of frequency. The water of hydration starts to absorb, according to this graph, at 50 MHz and reaches saturation values at 2 GHz. The range of free water absorption starts at about 0.8 GHz and rises exponentially. Above 4 GHz, free water absorption is predominant.

The basis for the special interest to the chemist – engaged in crystallization processes – in the different reaction mechanisms of the free and the bound water with respect to energy absorption, stems from the different functioning of water molecules in saline solutions in general, and in particular, in supersaturated solutions. Horne [8] maintains that ions dissolved in water form hydration spheres held by coulombic forces. Marcus [9] analysed the primary and secondary hydration spheres in terms of the thermodynamic functions of water activity. In the case of cations which bound the hydration spheres strongly, for instance calcium and magnesium, the hydration sphere is, as a rule, incorporated into a crystal formed in an aqueous solution.

In the present study an attempt has been made to examine the effect of microwave energy absorption during the precipitation of calcium phosphate species and to determine whether this energy can change the degree of hydration of calcium in the formed crystals, thus modifying their crystallographic structure.

The driving force for this research is summarized within the following paragraph [10].

Pure HA, however, may only be obtained in small quantities and only after using time-consuming (some months) and tedious methods. It is a great challenge to prepare large amounts of HA by precipitation within a few hours or days.

Hydroxyapatite powder is the basic material for the preparation of apatite ceramics which are currently

the subject of numerous material science studies. Apatite ceramics are considered the most biocompatible synthetic hard tissue material available and most likely candidates for use as bone and tooth prostheses.

2. Experimental

2.1. Materials

Stock solutions of calcium chloride, sodium dihydrogen phosphate and diammonium hydrogen phosphate were made from AR reagents. A microwave Oven, 1200W, 2450 MHz was used.

2.2. Procedure

Two sets of experiments in the microwave oven were carried out: (a) Starting with ionic solutions to precipitate calcium phosphate species, (b) using a preformed wet solid.

(a) To prepare the suspensions, equal volumes of 10 mM calcium and 6 mM phosphate solutions were simultaneously mixed in a chemical beaker. The ensuing suspension was introduced into the microwave oven for a period of 5 min. During this time the volume was reduced to half its initial value. This corresponds to increasing the concentrations of the calcium and phosphate to ca. 20 mM and 12 mM, respectively. The solid phase was separated by filtration and subjected to X-ray analysis.

(b) 1. The solid from experimental procedure (a) was filtered out and without further drying was introduced into the microwave oven.

2. Amorphous calcium phosphate (ACP) was suspended in water and the suspension was introduced into the oven.

3. ACP was suspended in basic buffer (pH 10) and introduced into the oven.

All samples were subjected to X-ray and infrared (i.r.) analyses.

3. Results and discussion

The definition of degrees of hydroxyapatite crystallinity differs markedly when applied by crystallographers and mineralogists as opposed to the definition by biologists, especially with reference to bone and dentine deposits. In our previous paper [11] a suggestion was put forward to define the degree of crystallinity according to the resolution of the characteristic peaks of the 2 1 1, 1 1 2, 3 0 0 and 2 0 2 planes reflections. Briefly, the categories were defined as follows:

1. No peaks at all; referred to as an amorphous phase.

2. A very wide peak containing all the reflections mentioned above and the reflection of the 0 0 2 plane.

3. Appearance of roughly defined peaks of all the mentioned planes and some weak indications of the 2 1 0 and 1 0 2 reflections.

4. Relatively well crystallized apatite.

These four categories will be used along with the appropriate diffractograms to characterize the results obtained in the present study.
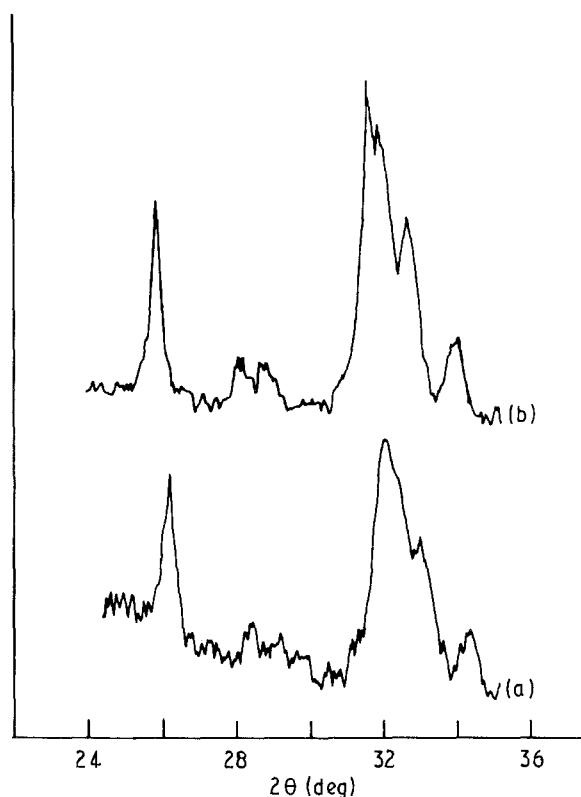

Figure 1 (a) The X-ray diffractogram of the solid precipitated under microwave irradiation from initially clear solution; (b) enhanced improvement by irradiation of the solid.

The X-ray diffractogram of the solid from (a) is presented in Fig. 1a. The degree of crystallinity can be classified slightly above class 3, giving good 0 0 2 reflection, slight 1 0 2, well shaped 2 0 2 reflection and visible shoulders of the 3 0 0 and 1 1 2 reflections on the pronounced 2 1 1 peak. Calcium phosphate precipitated from solutions with comparable concentrations (18 mM calcium and 10.8 mM phosphate) yielded precipitates with crystallinity of degree 1 or 2, i.e., either completely or nearly amorphous [9]. Thus there is an indication that the field of microwave energy can considerably speed up the maturation of amorphous phosphate to apatite in the solution within very short periods. Experiment (b)1 consisted of introducing the solid obtained in (a) and irradiating it with microwave for additional 15 min. After irradiation, the solid matured to well developed apatite as can be judged from the diffractogram in Fig. 1b. All the reflections from the relevant planes form well defined peaks.

It is well established that the addition of magnesium ions to a crystallizing calcium phosphate affects its crystallinity rendering it amorphous at certain compositions [12]. In (b)2, a completely amorphous sample of magnesium containing calcium phosphate was suspended in aqueous medium. The suspension was irradiated by microwave energy for 10 min. The X-ray diffractogram showed that no transition into apatitic crystal took place, the solid phase retaining its pronounced amorphous character. However, when the same amorphous solid was suspended in a basic buffered solution of pH = 10 and submitted to irradiation for 10 min, the separated crystals attained a degree classed between 2 and 3 as displayed in Fig. 2.
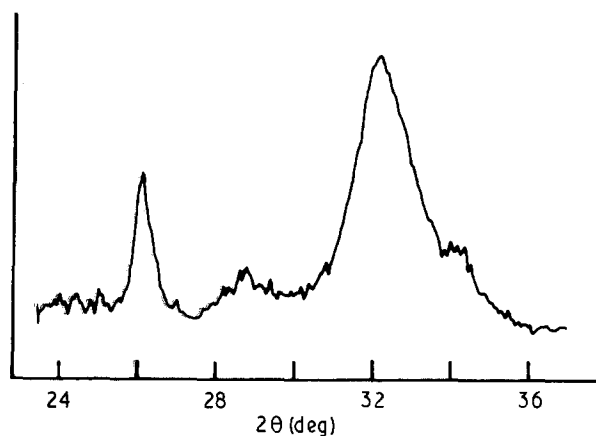
Figure 2 X-ray diffractogram of the Mg containing ACP after irradiation by microwave in basic pH = 10 medium.

Figure 3 Fourier transform infrared transmittance spectra of the solid phase (procedure (b)).

The sequence of procedures (a) and (b)1 was repeated with only a slight variation: 10 mm calcium and 6 mm phosphate solutions were mixed, subjected to microwave irradiation for 10 min; the solid was filtered out and irradiated for an additional 10 min. The X-ray analysis yielded a diffractogram of well developed apatite equivalent to that of the category 4. The crystallinity of category 4 was obtained in the previous study only with fluoroapatite. The i.r. spectrum also indicates that the hydroxyapatite is of satisfactory quality as evidenced by the triplet of peaks at 632, 601 and 565 cm^{-1} (Fig. 3).

The results show that the absorption of electromagnetic energy in the microwaves range does not convert a wet amorphous calcium phosphate solid to apatite (procedure (b)2). Only when an overwhelming amount of hydroxyl species is readily available (procedure (b)3), does a partial conversion occur. However, when the starting point is a system of ions in solution the microwave irradiation is directed toward the precipitation of crystals with recognizable apatitic character (procedure (a)).

When such a solid is subjected again to microwave irradiation, the degree of crystallinity is significantly improved (procedure (b)1).

Drying and heating of chemical products by microwave energy is an accepted technique in industrial applications. In this context the speed of attaining high temperatures and the efficiency of drying are of prime importance. It seems, however, that the present study transcends heating by convection.

The microwave energy supplied in the present experiments was 2450 MHz, i.e., 2.45 GHz. At this range the bound water appreciably absorbs the electromagnetic energy and the absorption by free water is minimal. It is true that the terms bound water and free water were defined for biological systems [7], but it is obvious that free water means very nearly the same in a biological system and in an aqueous solution, namely a loose ice-like three-dimensional network of hydrogen bonded water molecules in hexagonal rings, without being closely attached to any foreign molecule, ion or complex. The effect of the microwave energy on such a body of water will be merely that of heating thus enhancing evaporation. It may be assumed that
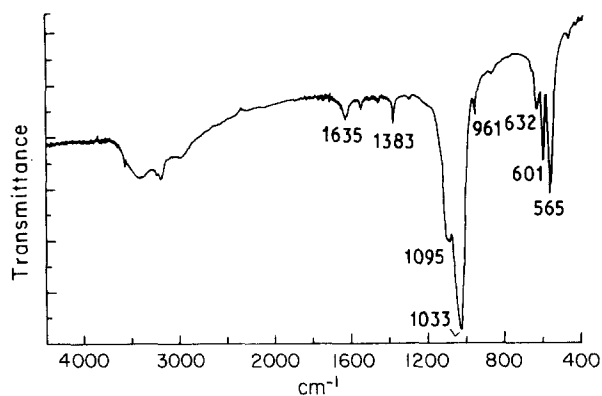
the bonds in the sphere of hydration of an ion are in the same order of magnitude as the bonds between a water molecule and an organic molecule or complex. If the latter bonds absorb the electromagnetic energy at the 2 GHz frequency, the bonds in the hydration sphere of calcium should also be affected. Whereas energies above 4 GHz may increase the translation energy a sufficient amount to cause rapid evaporation, the energy in the 2 GHz range may increase the rotational energy, thereby strongly affecting the bounded water. This input of energy can easily cause weakening of the bonds between the cation and its hydration sphere, facilitating the de-aquation step which is of paramount importance for the direct formation of apatite in aqueous solutions.

When CaP is precipitated from a basic aqueous solution, three main solid phases can be formed: ACP, OCP and HA ascending in thermodynamic order and descending on the time scale [13]. We believe, in agreement with the well known published theory of Christoffersen and Christoffersen [14] on crystal growth that: "growth units (ions) constantly bombard the crystal surface and are attracted by the electric charges of the ions on the surface. Therefore an adsorption layer is being formed and constantly renewed. The ions are hydrated in solution and must give off some of their hydration water molecules in order to get close to the ions already integrated on the surface. As the water molecule leaves the ion, it passes through an activated state, and the needed activation (Gibbs, or free) energy is several times the thermal energy (kT) . . . . Therefore, the adsorption rate may be controlled by the partial dehydration of the cations or by the penetration through the hydration layer of the crystal" [15].

In the formation of nuclei and crystal growth, the dehydration of the water bonded ion, is a time and energy consuming stage and critical one as well. Even from a stereogeometric aspect, it seems that dehydration of the ions is a critical stage in the formation of the nuclei of the growing crystal. We believe that this stage contributes the largest part of the energy needed to overcome the energy barrier (*Ea*) for the nuclei formation and crystal growth.

This may explain why ACP is first to precipitate, because the formation of a solid containing the hydration sphere, like the ions have in solutions, requires

much less Ea than the anhydrous product and can be referred as the kinetic product, while the anhydrous product can be referred to as the thermodynamic product.

Processes that affect the hydration sphere by weakening the water bonding or diminishing it by means of a chemical agent like alcohol which modifies water structure [16] or by adsorbing direct energy in the proper frequency (microwave) will therefore lower the Ea in such a way that will give preference to the anhydrous, more thermodynamically stable species, over the thermokinetic multihydrate species.

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