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Punctual investigation of surface sites of HA and magnesium-HA

L. Bertinetti[a,b,*], A. Tampieri[c], E. Landi[c], G. Martra[b], S. Coluccia[b]

[a] ISTEC-CNR, Str. delle cacce 37, 10100 Torino, Italy
[b] Dipartimento di Chimica IFM and NIS Centre of Excellence, Università degli studi di Torino, V. Giuria 7, 10100 Torino, Italy
[c] ISTEC-CNR, V. Granarolo 64, 38018 Faenza, Italy

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Abstract

IR spectroscopy of adsorbed CO is used for the first time to characterize surface cationic centres of pure and magnesium doped hydroxyapatite (HA and MHA, respectively). In air, water molecules saturate the coordination sphere of exposed surface cations by a strong coordinative interaction. At first, the materials were outgassed at 300 °C in order to remove water molecules adsorbed on the surface. Following this treatment, surface sites able to adsorb CO are produced. The vibrational frequency of adsorbed CO is sensitive to the features of the adsorbing sites. The IR spectra of CO adsorbed on HA revealed the presence of surface Ca^{2+} ions differing for the degree of coordinative unsaturation and of P–OH groups. On MHA, CO was able to monitor the presence of surface Mg^{2+} ions, the amount of which increased by aging the material in air.

Keywords: Surfaces; Apatite; Magnesium; Spectroscopy; Biomedical applications

1. Introduction

Calcium hydroxyapatite (HA) is the structural model for the inorganic phase of the bone tissue. The HA lattice can easily accommodate a variety of substituents both cationic and anionic, inducing modifications of the crystallinity, morphology, lattice parameters and stability of the apatite structure. This feature can be used as a powerful tool to prepare materials with specific characteristics mimicking the biological apatites, which are non-stoichiometric and contain structural imperfections and defects as well as foreign ions,[1,2] increasing the level of structural instability. Among substituting cations, magnesium is widely studied due to its biological relevance. It has been verified that in calcified tissues, the amount of magnesium associated with the apatitic phase is higher (about 5 at.%) at the beginning of the bone remodelling process and decreases with increasing calcification.[3] It is already known that magnesium increases the nucleation kinetic of HA contemporary retarding that of its crystallization; for that reason magnesium concentration is higher in the cartilage and in young bone and there is growing evidence that it may be an important factor in the qualitative changes of the bone matrix that determines bone fragility. Magnesium depletion adversely affects all stages of skeletal metabolism, causing cessation of bone growth, decrease of osteoblastic and osteoclastic activities and osteopenia. Consequently, the incorporation of magnesium ions into the hydroxyapatite structure is of great interest for the developing of artificial bone.[4–6] This study would provide a first description of surface modification and ageing stability of magnesium hydroxyapatite, subsequent to the incorporation of Mg^{2+}, by mean of IR spectroscopy of adsorbed molecules. Such technique, extensively described elsewhere,[7] has been successfully employed for evaluating the reactivity and the surface electronic states of a wide variety of materials,[8] and is very promising also for biomaterials.

2. Experimental

Pure hydroxyapatite powder (BET surface area = 78 m^2/g), HA, and magnesium substitute apatite (BET

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* Corresponding author. Tel.: +39 011 3977233; fax: +39 011 346288.
E-mail address: l.bertinetti@to.istec.cnr.it (L. Bertinetti).

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surface area = 74 m^2/g), MHA, with nominal composition $(Ca_{1-x}Mg_x)_5(PO_4)_3OH$, with $x = 0.15$, were synthesized through an aqueous medium procedure, starting from calcium hydroxide, ortophosphoric acid and magnesium chloride (Sigma–Aldrich), whose details are reported elsewhere.[9] The chemical composition of the samples were determined by ICP spectrometer, revealing that total amounts of Mg actually entered in the HA lattice is 50% in respect to the total introduced in the synthesis process, i.e. 8% at. In the next section we will refer to the fresh sample as MHA-f and to the sample stored in air at 20 °C for 2 months as MHA-2m. X-ray powder diffractometer (Rigaku–Minifiex) with Cu Kα radiation was used at the operating conditions of 33 kV and 25 mA. The XRD data were collected over 2θ range of 10°–60° at step size of 0.02°. For infrared spectra, in order to enhance the absorption by surface species, otherwise too weak, HA and MHA powders were prepared in form of a self-supporting pellet. The samples were placed in a quartz IR cell equipped with KBr windows properly designed to carry out spectroscopic measurements both at room temperature (rt) and liquid nitrogen temperature (77 K). The cell was connected to a conventional vacuum line (residual pressure: 1×10^{-3} mbar) allowing all thermal treatments and adsorption–desorption experiments to be carried out in situ. FTIR spectra, were obtained using a Bruker Vector 22 spectrometer (resolution: 4 cm^{-1}) equipped with an MCT detector. The spectra of adsorbed molecules are reported in absorbance, after subtraction of the background spectra of the samples before adsorption. High purity CO was employed without any additional purification except liquid nitrogen trapping. Before adsorption of CO, samples were outgassed at 300 °C for 1 h.

3. Results and discussion

Fig. 1a shows the IR spectrum of HA sample in air. This sample exhibits a strong and sharp band out of scale at 3572 cm^{-1} emerging from a very broad and intense band at 3700–2500 cm^{-1}, a band at 3550 cm^{-1}, a band and at 1642 cm^{-1}, and a complex set of absorptions with two peaks at 1475 and 1415 cm^{-1} accompanied by a number of ill-defined shoulders. The 3572 cm^{-1} band is assigned to the stretching mode ν_{OH} of structural hydroxyls groups in the bulk of microcrystals and the intense and broad band covering the range 3700–2500 cm^{-1}, is associated to the stretching modes of adsorbed water molecules. The band at 3550 cm^{-1} is still unassigned in literature; we propose that it is due to some vibrational manifestation of hydroxyls in the bulk of the material, as it does not shift after deuteration at room temperature, likely arising from the coupling of OH^- oscillators. The band at 1642 cm^{-1} is assignable to the bending mode of molecular water adsorbed on the hydroxyapatite surface. Finally the bands in the 1360–1560 cm^{-1} range are due to the stretching modes of groups present as structural carbonates entrapped in the lattice of the hydroxyapatite as a consequence of dissolution of CO_2 from air during the synthesis process and/or adsorbed on the surface from the air. The range below 1250 cm^{-1}, is totally obscured by the intense absorptions of the phosphate groups. By outgassing at rt for 1 h (Fig. 1b) molecularly adsorbed water is largely eliminated as shown by the decrease of the intensity of the broad absorption at 3700–2500 cm^{-1} and of that at 1642 cm^{-1}. Residual water molecules are progressively removed as the outgassing temperature is increased up to 200 °C (Fig. 1c and d) with the last vestiges of the stretching and bending bands disappearing at 300 °C (Fig. 1e). Following above treatments, the ν_{OH} band of structural hydroxyls at 3572 cm^{-1} and those at low frequencies due to carbonate groups do not change significantly. Moreover, thanks to the disappearing of the overwhelmingly absorption due to molecular water, three bands become evident, though weak, on the high frequency side of the dominant sharp band at 3572 cm^{-1}. Such bands in the 3700–3600 cm^{-1} range are assigned to the OH stretching vibration of P–OH surface groups.[10,11] Considering the overall evolution of the spectra in Fig. 1 the decrease of the broad band at 3700–2500 cm^{-1} and water bending band indicates that physisorbed multilayered liquid-like water was progressively removed from the system as a consequence of mere pumping off at room temperature. A fraction of water, associated with the residual absorption at 1642 cm^{-1} and with the broad tail at 3700–2500 cm^{-1} experiences a stronger interaction with the material (Fig. 1b). These water molecules are progressively removed by increasing the outgassing temperature up to 300 °C, as shown by the gradual disappearance of such bands (Fig. 1c–e). According to the well known behaviour of most oxides,[12] such strongly held water molecules are chemisorbed on the surface acidic cationic sites to form $Ca^{2+} \cdots OH_2$ adducts. Vestiges of molecular water eliminated at the highest outgassing temperatures (Fig. 1e) might well come from the bulk. The slight decrease, if any, of structural OH^- band at 3572 cm^{-1} upon outgassing at 200 and 300 °C (Fig. 1d and e) indicates that some dehydroxylation process occurs, resulting from condensation or from proton migration. Once surface cationic centres have lost the by water molecules which saturated their coordination sphere, we

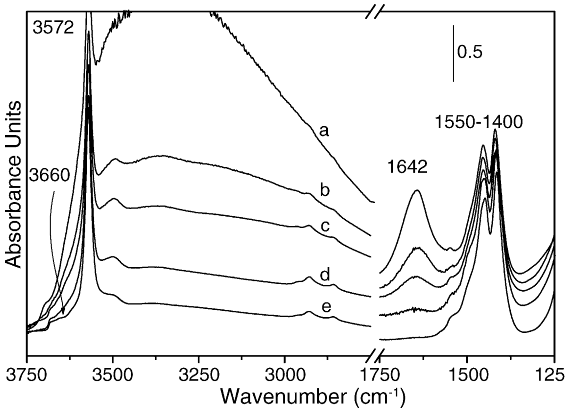

Fig. 1. IR spectra of HA: in air (a); outgassed at rt (b) for 1 h; 100 °C (c) for 1 h; 200 °C (d) for 1 h; and 300 °C (e) for 1 h.

can have access to atomic information on their local structure studying the modification of vibrational features of adsorbed molecules. CO is known to be a good probe for cationic centres, as adsorption occurs on surface Lewis acid sites and produces IR bands at frequencies higher than the stretching frequency of the free molecule in the gas phase (2143 cm^{-1}). The stretching frequency increases with the polarizing field, which is strongly correlated with coordinative unsaturation and with the charge/radius ratio of adsorption site. In the case of HA and MHA outgassed at 300 °C, adsorption sites for CO at 77 K are Mg^{2+} and Ca^{2+}; at low temperature, also P–OH groups can stabilize CO groups. Spectra of CO adsorbed at 77 K on the surface of HA pre-outgassed at 300 °C are reported in Fig. 2. At high CO coverage (15 mbar) a band, highly asymmetric on its low frequency side, is observed in the CO stretching region. As the coverage decreases by lowering the pressure of the gas phase down to 0.05 mbar, the intensity progressively decreases and the maximum shifts from 2170 to 2180 cm^{-1}. Below 0.05 mbar CO stretching band decreases and shifts further to 2182 cm^{-1}. It has to be noticed that, as a consequence of adsorption of CO, P–OH stretching band is reduced in intensity and, while decreasing the pressure of carbon monoxide, it is progressively restored (Fig. 2 inset). At high coverage, CO interacts with all acidic surface species and, consequently, the band at 2170 cm^{-1} is the superimposition of a number of components assignable to CO interacting with hydroxyls and Ca^{2+} cations exposed at the surface. The lower is the frequency of CO stretching, the lower the polarizing field of the adsorption site and the strength of its interaction with this probe molecule. As CO adsorbed at 77 K onto the sample simply pre-outgassed at rt (Fig. 2 dashed line), exposing at its surface basically P–OH groups and water hydroxyls, produces an IR band in the range 2145–2170 cm^{-1}, the components at lower frequencies (2150–2170 cm^{-1}) of carbon monoxide adsorbed on the HA are assignable to the CO adsorbed on the surface hydroxyls, whereas those at higher frequencies (2160–2182 cm^{-1}) have to be assigned to CO adsorbed on Ca^{2+} sites exposed at the surface. The latter component arises from adsorption of CO on a large number of Ca^{2+} ions with heterogeneous chemical features difficult to separate in specific sub-components. Two main reasons generate such surface heterogeneity for Ca^{2+} ions: one is associated with the presence of ions differing for the coordination, the second resulting from the location of such ions in different surface structures. The spectra of adsorbed CO at higher coverage currently could not give defined information on the structure of Ca^{2+} sites, but can provide "average" information on the strength of the electric field generated by surface cationic centres. The crossing of the band at about 2180 cm^{-1} with the main band at 2170 cm^{-1}, observed when decreasing coverage, are due to fadeing away of dynamic and static adsorbate–adsorbate interactions among CO oscillators[13,14] and/or to the transformation of dicarbonylic adducts $Ca^{2+}\cdots(CO)_2$, due to the CO adsorbed on a Ca^{2+} ions coordinatively most, unsatured, in monocarbonylic ones, as reported in literature for MgO and other oxides.[15,16] Anyway, the most stable species still present at the lowest coverage, band at 2182 cm^{-1} in Fig. 2e, are certainly adsorbed on the surface Ca^{2+} sites with the largest coordinative unsaturation. Spectra of CO adsorbed on the hydroxyapatite containing 8 at.% of magnesium, MHA-f, compared with those relative to unexchanged HA (same spectra of Fig. 2) are reported in Fig. 3. Though the spectra related to the two samples appear very similar, it has to be noticed that the stretching band of CO adsorbed on the MHA systematically shows weak components on the high frequency tail (see arrow in Fig. 3 section A). Such new components become clearly observable at very low coverage (section B of Fig. 3), at about 2200 and at 2210 cm^{-1}. These bands can undoubtedly be assigned to CO adsorbed onto the newly introduced Mg^{2+} substituting Ca^{2+} in pure hydroxyapatite. Indeed, as Mg^{2+} ions have a higher charge/radius ratio then the Ca^{2+}, the new bands observed in the case of MHA-f, 2210 and 2200 cm^{-1}, due to surface $Mg^{2+}\cdots CO$ adducts are at higher wave-number. Such high frequencies are very similar to those of CO adsorbed by sites with the lowest coordination in MgO[17] ($Mg_{3c}{}^{2+}$). The low intensity of the bands at 2200–2210 cm^{-1}, compared with that at 2182 cm^{-1}, reflects the low concentration of Mg^{2+} in this sample is (8 at.%). It is appropriate to recall that XRD data indicate that the presence of magnesium at concentrations up to 15 at.%, Mg^{2+} do not induces formation of any different phase than hydroxyapatite.[9] It has been shown above that infrared spectra of adsorbed CO are very sensitive to the nature

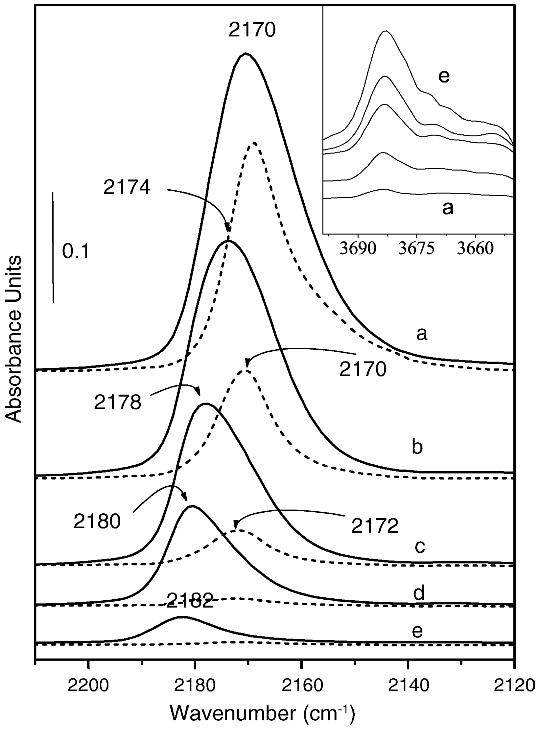

Fig. 2. IR spectra of adsorbed CO at 77 K on the HA outgassed at rt (dashed line) and preoutgassed at 300 °C (solid line) at decreasing coverage: 13 mbar (a); 0.5 mbar (b); 0.1 mbar (c); 0.05 mbar (d); and 0.01 mbar (e); in the inset is reported the 3700–3650 cm^{-1} range for HA outgassed at 300 °C.

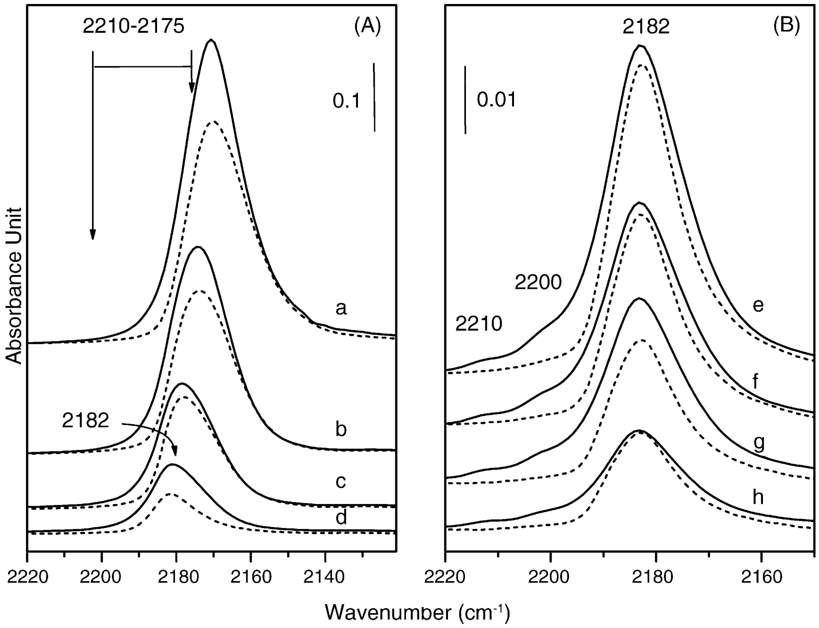

Fig. 3. IR spectra of adsorbed CO at 77 K on the HA (dashed line) and MHA-f (solid line) at decreasing coverage: from 13 mbar (a) to 0.04 mbar (d) in section A and from 0.03 mbar (e) to 0.001 mbar (h) in section B.

of surface sites. We might then expect that modification of surface composition due to a number of factors might be revealed by such analysis. Ageing is one of the most relevant processes in biomaterials, and, in the case of hydroxyapatite is believed to be accompanied by migration of cations, and, possibly, by Mg^{2+} enrichment to the surface. Fig. 4 compares the spectrum of CO adsorbed (low coverage) on a fresh MHA sample with the spectrum of CO adsorbed on the same sample aged for 2 months (MHA-2m) at ambient conditions. Despite the spectra of adsorbed CO on the two samples are similar, observable differences, though small, are meaningful. In fact, the intensity of the two components at 2210 and 2200 cm^{-1}, is somewhat increased in the case of the sample MHA-2m, suggesting that the concentration of Mg^{2+} ions at the surface is larger on the aged sample. This behaviour suggests that MHA is not a stable material when exposed at the air, with a tendency of the Magnesium to gradually segregating on the surface. These data well agree with structural parameters of aged magnesium substituted hydroxyapatities obtained by XRD: during the aging at room temperature, expecially if the powdered samples have high specific surface, lattice parameters are found to increase gradually up to restore the values typical of pure HA, indicating that the Magnesium substituting Calcium in HA structure is progressively expelled: the phenomenon is completed in 2–3 months without the detectable formation of any new crystalline phase. IR spectroscopy of adsorbed molecules cannot, alone, provide a full description of the aggregation state of magnesium at the surface. Studies are in progress in order to determinate the aggregation state, if any, of magnesium at the surface and to investigate which is the driving force for the migration of magnesium towards the surface; it can be hypothesized that water adsorbed on the surface of such material and/or occluded in the crystal[18] can play a crucial role in this process.

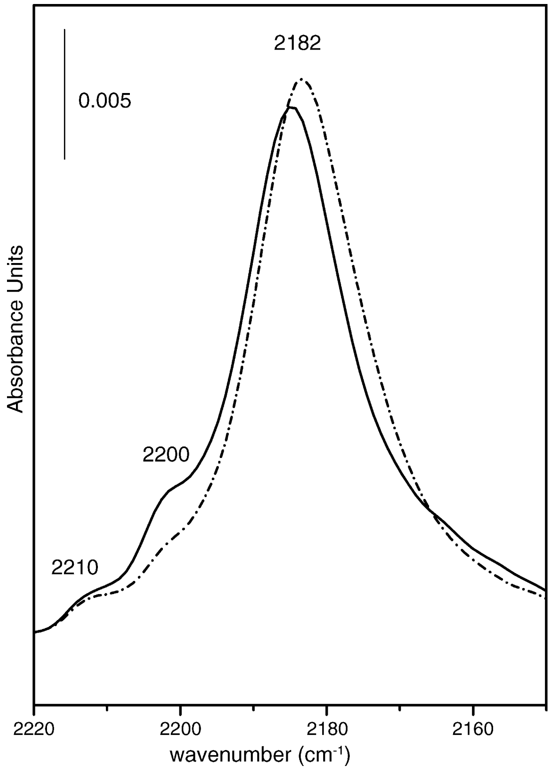

Fig. 4. IR spectra of adsorbed CO on the MHA-f (dashed line) and MHA-2m (solid line) at 0.001 mbar.

4. Conclusion

IR. spectroscopy of adsorbed CO is revealed as a powerful technique to recognize cationic centres exposed at the surface of biomimetic apatites and to follow their evolution during time. Hydroxyapatite exhibits flat surfaces and a great heterogeneity of Ca^{2+} ions exposed at its surface differing for their local structure. Inclusion of magnesium in the lattice of hydroxyapatite enhances the mean electric field at the surface of the material, likely inducing relevant changes in surface properties. Moreover, a migration process of Mg^{2+} ions from the bulk towards the surface is observed after 2 months, indicating that such material prepared by precipitation in aqueous solution and stored in air is unstable; this aspect could play a significant role in synthesis and storage strategy of this type of biomimetic apatite.

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