



Stabilization of hemoglobin in double layered hydroxides to be used in carbon monoxide bio-oxidation I-synthesis and characterization



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ABSTRACT

Carbon monoxide is a very toxic compound and, then, its oxidation to carbon dioxide is of great importance. Many heterogeneous processes have been proposed, but a few consider a mimetic approach. Hemoglobin molecules have high affinity to gases as CO or CO₂; therefore, if they are retained they should work as an efficient retainer and catalyst. Being basic they may be anchored in hydrotalcites. The obtained hemoglobin-hydrotalcite hybrid material may, then, promote the capture of CO and, thereafter, oxidate it to CO₂.

In this work, hydrotalcites containing either aluminum or iron were prepared through three different procedures and tested in hemoglobin retention. In each of those syntheses, hybrids prepared in presence or not of microwave irradiation are compared. The solids were structurally and morphologically different. The sample prepared through the adsorption method presented the strongest interaction between organic and iron inorganic entities. The redox properties of iron explain that strong interaction. Such is not the case in aluminum containing hydrotalcites.

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1. Introduction

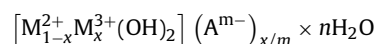
The oxidation of carbon monoxide is of great importance because it is extremely toxic. Many heterogeneous processes exist to oxidate CO to CO₂. The first step is to immobilize or retain CO [1]. Several adsorbents such as titania based catalysts, alumina based materials or CeO₂ composites [2,3], among others, have been proposed. Nevertheless, not many are inspired in the “natural” process which turns out to be most efficient in the animal bodies whose blood, in fact hemoglobin present in red blood cells, transports gases such as oxygen or carbon dioxide. Still, hemoglobin may be poisoned due to a preferential association with gases such as carbon monoxide. Indeed, the affinity of hemoglobin for CO, *in vivo*, is roughly 200–225 greater than that for oxygen.

In a first step one could imagine the immobilization of CO on hemoglobin; and, in a second step, its oxidation to CO₂. However red blood cells containing hemoglobin are suspended in blood plasma, their use is difficult. In this work, we propose to anchor hemoglobin, which is a protein, with basic character on a solid to use it as a heterogeneous catalyst to oxidize CO. The main advantage of the proposed procedure is to incorporate hemoglobin into

a solid to obtain a hybrid compound whose management should be easy. CO should be retained on the immobilized and stabilized hemoglobin and then the CO oxidation process becomes a heterogeneous catalysis problem.

Although in previous works, it has been shown that iron porphyrins, myoglobin, and hemoglobin can be retained in Mg–Al double layered hydroxides [4–7], other compositions may turn out to be more performing due to the oxidation-reduction properties of elements like Fe or Co [8,9]. Indeed, the charge transfer could be crucial. To retain hemoglobin which is a protein with a very low charge density, a metallic cation hydrotalcite whose electronegativity value is low should be more efficient. Thus, as Al³⁺ and Fe³⁺ differ in their electronegativity values, 1.499 and 1.687 respectively, Mg–Al and Mg–Fe hydrotalcites are expected to present a different behavior in hemoglobin retention. The electron transfer is expected to be from magnesium to aluminum in lower degree than magnesium to iron [10,11].

Double layered hydroxides, more specifically hydrotalcite-like compounds or simply hydrotalcites, are anionic clay-like compounds. Their chemical formula is:



where x di-valent M²⁺ cations may be replaced by tri-valent M³⁺ cations, which produce positively charged layers. This charge is

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Table 1

Comparison of interlayer distance, specific surface area and pore size distributions of the reference samples and hybrids prepared by the regeneration, simultaneous and adsorption methods.

Sample	Interlayer distance (Å)*	Specific surface area (m ² /g)	Main peaks in the pore size distributions (nm)
Al–MW; Fe–MW	8.4; 8.4	9.3; 21.0	3 and 7; 5 and 20
Al–C; Fe–C	8.3; 8.4	15.0; 58.1	3 and 6; 3 and 14
R–HbAl–MW	8.3	17.1	3
R–HbAl–C	8.3	17.4	3
R–HbFe–MW	8.7	4.1	3 and 10
R–HbFe–C	8.7	11.3	3 and 40
S–HbAl–MW	8.3	3.1	3
S–HbAl–C	8.3	4.2	3
S–HbFe–MW	8.3	12.6	5 and 40
S–HbFe–C	8.3	8.6	5 and 30
A–HbAl–MW	8.3	5.8	3
A–HbAl–C	8.3	6.3	3
A–HbFe–MW	8.4	46.4	3 and 8
A–HbFe–C	8.4	89.2	3 and 8

neutralized by x/m anions A^{m-} as CO_3^{2-} , SO_4^{2-} , Cl^- or NO_3^- , among others. The most frequent divalent metals are those whose ionic radii vary between 65 pm (Mg) and 80 pm (Mn) whereas the radii of the trivalent metal has to be comprised between 50 pm (Al) and 69 pm (Cr). The nature of the couple divalent–trivalent metal is most relevant [12–14].

Hydrotalcite synthesis method has been significantly improved substituting the conventional hydrothermal step by a microwave irradiation exposure [15–17]. The long crystallization time is substantially reduced. In the microwave irradiated synthesis, correlations between the features of the obtained hydrotalcite-like compound and the anion and cation nature has not been established or carefully studied. For instance, in NiMg/Al–hydrotalcites synthesized in presence of microwaves, we have found that magnesium and aluminum are not homogeneously distributed: aluminum and nickel are selectively retained in the hydrotalcite particle core [18]. Morphology may be altered through the use of templates such as hair, nylon or cotton fibers [19]. The understanding of those parameter effects should provide a control on the structural and morphological characteristics of the materials.

In this work, the effect of iron in hydrotalcites to retain hemoglobin is studied and compared to the corresponding results obtained with Mg–Al hydrotalcite. On the one hand, the hydrotalcite samples are synthesized through: (a) the conventional method, or (b) in presence of microwave irradiation in the crystallization step.

Hemoglobin retention was performed, following three procedures: (i) using the regeneration property of hydrotalcite, *i.e.* the hydrotalcite network reconstruction from the corresponding mixed oxides obtained after calcination at 500 °C. (ii) hemoglobin was also immobilized straightforward after contact with the solid. (iii) lastly, hemoglobin was mixed with hydrotalcite precursors before hydrotalcite crystallization.

2. Experimental

2.1. Synthesis

2.1.1. Materials

Aluminum, magnesium and iron (III) nitrate, from Sigma–Aldrich (98%, 99 and 98% purity), were used as reactants to synthesize hydrotalcites. Sodium hydroxide (Baker, 99.5% purity) was the precipitating agent. Bovine hemoglobin (MW = 64500 g/mol) was provided by Aldrich.

2.1.2. Microwave assisted samples

2.1.2.1. *Synthesis by structural regeneration (memory effect).* The Mg/Al hydrotalcite reference sample, labeled Al–MW, was synthe-

sized from Mg- and Al-nitrate aqueous solution (1 M) and sodium hydroxide solution (1 M). The flow of each solution was adjusted to maintain a constant pH of 9. The amounts correspond to a molar ratio Mg/Al of 4. The resulting precipitate was treated in a microwave autoclave (MIC-I Sistemas y Equipos de Vidrio S.A. de C.V.) at 80 °C and 200 W for 10 min. The microwave equipment consists of a glass reactor of 500 mL, which fits in a microwave oven whose power may be selected. A stirring mechanism is adapted to the reactor to maintain constant temperature all over; temperature is controlled through an infrared radiation sensor [18]. The solid was recovered by decantation and washed with distilled water up to a pH value of 9 and dried in an oven at 70 °C overnight. Mg/Fe hydrotalcite, reference sample (Fe–MW), was synthesized from Mg- and Fe (III)-nitrate aqueous solution (1 M) and sodium hydroxide solution (1 M) at the conditions described for Al–MW sample.

The Mg/Al and Mg/Fe hydrotalcites were calcined at 500 °C for 5 h and 3 g of the resulting oxides were dispersed with distilled water (150 mL) under stirring. Then, 2.5 g of hemoglobin (Hb) were slowly added. Both slurries were maintained in stirring at room temperature for 24 h, in N₂ inert atmosphere. The solids were washed several times with distilled water, recovered by centrifugation and dried at 70 °C for 24 h. The resulting samples were labeled R–HbAl–MW and R–HbFe–MW for aluminum and iron containing hydrotalcites, respectively.

2.1.2.2. *Synthesis by simultaneous precipitation.* To obtain 5 g of hydrotalcite, a 1 M solution containing magnesium and aluminum nitrates (12.1 and 4.5 g, respectively) was mixed drop-wise simultaneously with a 1 M solution of sodium hydroxide (4.7 g) and with a dispersion of hemoglobin (2.5 g in 150 mL of water), maintaining a constant pH of 9. The reactant amounts correspond to a hydrotalcite molar ratio Mg/Al of 4. The slurry was treated at 50 °C for 10 min in the microwave autoclave described above. After this hydrothermal treatment, the solids were washed several times, recovered by centrifugation and dried at 70 °C for 24 h, sample S–HbAl–MW. Another sample was synthesized as the previous one but using iron instead of aluminum nitrate, sample S–HbFe–MW.

2.1.2.3. *Synthesis by adsorption.* Mg/Al and Mg/Fe hydrotalcites were prepared as described above, reference samples Al–MW and Fe–MW. Three grams of the sample were dispersed in distilled water (150 mL) and stirred for 5 min. Then, 2.5 g of hemoglobin were slowly added, maintaining the stirring for 48 h. The solids were washed several times, recovered by centrifugation and dried at 70 °C for 24 h. The process was carried out in N₂ inert atmosphere and the resulting samples were labeled A–HbAl–MW and A–HbFe–MW.

2.1.3. Conventionally prepared samples

All the previous samples were prepared through the conventional method reported elsewhere [20], i.e., the crystallization step was carried out heating the sample precursors in an Erlenmeyer flask coupled to a cooling coil at 70 °C for 24 h. The obtained samples are labeled with C instead of MW. A diagram which summarizes all the preparations is presented in Fig. S1.

2.2. Characterization methods

2.2.1. X-ray diffraction

A Bruker-axs D8-advance diffractometer coupled to a copper anode X-ray tube was used to identify the compounds present in the powdered samples. A diffracted beam monochromator selected the $K\alpha$ radiation.

2.2.2. FTIR spectroscopy

FTIR spectra in the region 4000–400 cm^{-1} were obtained with a Magna-IR Spectrometer 550 Nicolet. The pellets were prepared with KBr.

2.2.3. Nitrogen adsorption

The BET surface areas were determined from the nitrogen adsorption–desorption curves by the conventional multipoint technique with a Micromeritics ASAP 2020. The pore size distribution curves were obtained applying the BJH method to the desorption branch. The samples were pretreated at 150 °C for 12 h at high vacuum.

2.2.4. Scanning electron microscopy

For SEM studies, a JEOL JSM-7600F (Scanning Electron Microscope) was used at various magnifications. The local elemental chemical composition was determined by EDS.

3. Results

3.1. Reference samples

For comparison purposes four reference samples were synthesized. They do not contain hemoglobin. The X-ray diffraction patterns of the samples Al-MW and Al-C as well as Fe-MW and Fe-C show that the initial samples, as expected, are crystalline and no other compounds than hydroxalcite are found. The interlayer distances, Table 1, determined by the 003 peak position, are 8.4 Å, characteristic of a hydroxalcite exchanged with nitrate and hydroxyl anions [14].

In SEM images the morphology of samples Al-C and Fe-C differ. In the first case, the surface is rather smooth and dense, the morphology seems to be layered, whereas, in the second, flakes (100–200 nm) agglomerate as small desert roses, in both, conventional or microwave irradiated samples. As expected, the specific surface area of sample Al-MW (9.3 m^2/g) is much lower than that of Fe-MW (21.0 m^2/g). Iron propitiates, then, a more open texture. In sample Al-MW, the pore size distribution is monomodal and centered at $D=3$ nm; in sample Fe-MW the pore size distribution is broad with maxima at $D=5$ and 20 nm.

The infrared spectrum of pure hemoglobin shows peaks at 3430 and 1100 cm^{-1} typical of OH groups corresponding to heme carboxylates. The peaks at ca. 2900 cm^{-1} and at 1300 and 1500 cm^{-1} may be attributed to CH_2CH_2 groups. The vibrations of $\text{C}=\text{N}-\text{C}$ group appear at 1650 cm^{-1} . The broad peak found at 650 cm^{-1} is due to heme iron [21]. The FTIR spectrum of non-containing Hb hydroxalcite, sample Al-MW, presents peaks at 3500, 1650, and 1400 cm^{-1} ; when compared with R-HbAl-MW sample, only the peaks at 2900 and 1500 cm^{-1} reveal the presence of hemoglobin, Fig. 1.

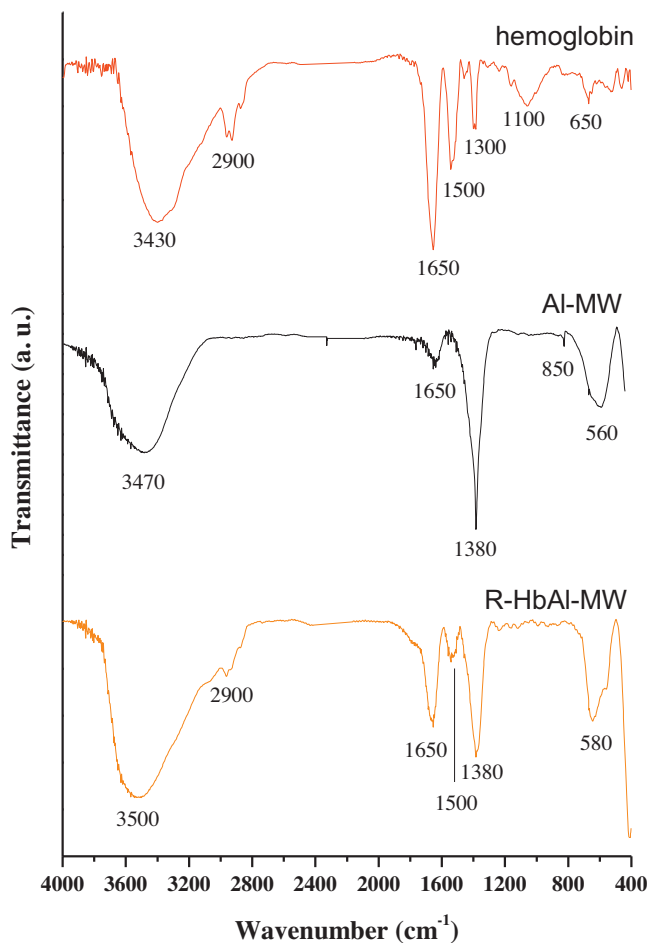


Fig. 1. Infrared spectra of hemoglobin, Mg/Al hydroxalcite, sample Al-MW and the hybrid obtained by regeneration, sample R-HbAl-MW.

3.2. Synthesis by structural regeneration (memory effect)

In Fig. 2 the diffractograms of samples R-HbAl-MW, R-HbFe-MW, R-HbAl-C and R-HbFe-C are displayed. The crystallinity of microwave irradiated samples differs depending on the M^{3+} cation. The R-HbAl-MW sample is crystalline with sharp peaks and no other compound than hydroxalcite is observed. The 003 peak location corresponds to an interlayer distance of 8.3 Å (Table 1), indicating that the hydroxalcite crystallizes independently of hemoglobin, which is not intercalated and does not intervene in the regeneration mechanism. Instead, in the Fe containing sample, R-HbFe-MW, a rather small amount of crystalline hydroxalcite is obtained, the large amorphous compound content, revealed by the broad peak from 5 to 60° (2θ), is attributed to a poorly ordered structure. The peaks of the iron containing samples are slightly shifted towards smaller angular values, probably due to a lower layer charge in spite of the low Mg/Fe ratio (Table 2).

If the samples are prepared through the conventional method, the X-ray diffraction patterns are similar, samples R-HbAl-C and R-HbFe-C, showing again that hydroxalcite is formed without hemoglobin participation.

The scanning electron microscopy images, at high and low magnification, of sample R-HbAl-MW are shown in Fig. 3. The sample is constituted by particles ca. 10 × 100 nm of well crystallized hydroxalcite in a house of cards arrangement at nanometric level, whereas at micrometric level rough layers ca. 0.3 × 3 μm can be observed. Most probably the roughness of the layers at micrometric level is due to the deposition of some hemoglobin. Hemoglobin, as well,

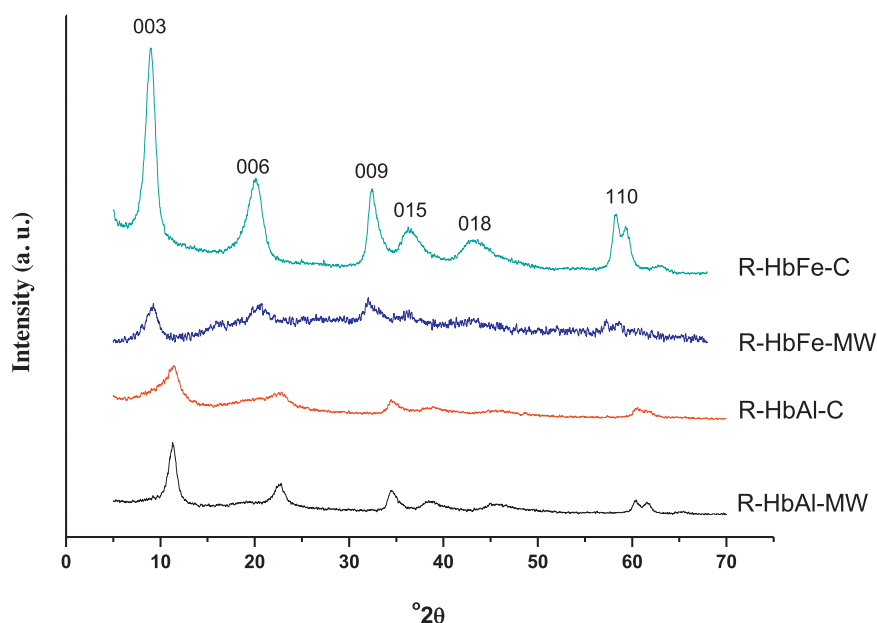


Fig. 2. Comparison of the X-ray diffraction patterns of R-HbAl-MW, R-HbFe-MW, R-HbAl-C and R-HbFe-C samples. Only the sample R-HbFe-MW shows a high amount of amorphous material.

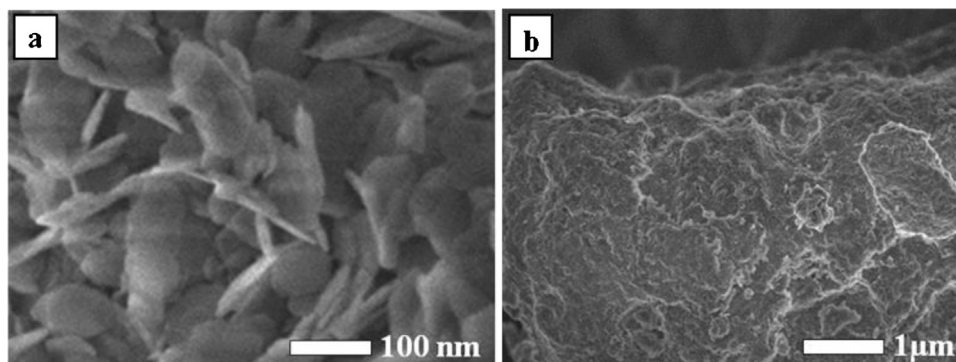


Fig. 3. Scanning electron micrographs of the sample R-HbAl-MW showing the morphology of hydrotalcite regenerated in presence of hemoglobin and microwave irradiation. The size of the nanometric layered particles is close to 10×100 nm. (a) $\times 100,000$ and (b) $\times 10,000$.

Table 2

Atomic ratios obtained by EDS analysis for samples prepared by the regeneration, simultaneous and adsorption methods.

Sample	Mg/(Al or Fe)	C/(Al or Fe)
R-HbAl-MW	3.6	6.2
R-HbAl-C	3.4	7.1
R-HbFe-MW	1.9	7.2
R-HbFe-C	1.6	5.3
S-HbAl-MW	3.1	20.0
S-HbAl-C	3.2	11.8
S-HbFe-MW	2.1	10.3
S-HbFe-C	2.7	6.1
A-HbAl-MW	3.5	18.6
A-HbAl-C	3.5	19.4
A-HbFe-MW	1.0	0.8
A-HbFe-C	0.9	0.6

distributed among the hydrotalcite particles can cause the disorder observed at nanometric level. Then, the folded hemoglobin (ca. 250 nm) works as a template to generate the inter-particle space, which explains the roughness observed on the external surface.

In sample R-HbAl-MW, the FTIR peaks at 2900 and 1500 cm^{-1} reveal the presence of hemoglobin, Fig. 1. The FTIR spectra of the other R-samples are similar to that of R-HbAl-MW, Fig. S2. Hence,

in all these samples hemoglobin is present and not denatured. This result is in agreement with EDS spectra where an intense peak attributed to carbon is observed in hemoglobin containing sample (R-HbAl-MW).

In Table 1 the specific surface areas and pore size distributions of the regenerated samples in presence of hemoglobin are compared. The specific surface area of the iron regenerated samples turns out to be smaller than the corresponding original samples; the R-HbFe-MW and R-HbFe-C areas are five times lower than the references Fe-MW and Fe-C. The regeneration process in presence of hemoglobin results in a material more compact, this feature is enhanced with microwave irradiation. The other way round, in Al containing samples it seems that hemoglobin disperse hydrotalcite lamellar particles and the nitrogen gas reaches a higher surface.

The elemental chemical composition obtained by EDS analysis (in mole%) of sample R-HbAl-MW is shown in Table 2. All samples present a high amount of carbon, which must be due to hemoglobin, as in FTIR spectra no carbonates were detected. Note that, to avoid carbonates formation, all hybrid samples were synthesized in nitrogen inert atmosphere.

The Mg/Al ratios of samples R-HbAl-MW and R-HbAl-C are 3.6 and 3.4; these values are lower than the nominal value of 4, showing that an amount of Mg^{2+} cations is lost during the

washing step. The iron containing samples present an Mg/Fe ratio two times lower than the nominal. It seems that a fraction of the Mg is substituted by Fe^{2+} , showing that iron oxidation state is not only +3. Magnesium probably is lost during the washing step. The ratio $C/(\text{Al or Fe})$ reveals the amount of hemoglobin molecules retained per M^{3+} cation. Hence, it means that the highest the value the more positively charged is the corresponding M^{3+} site. Furthermore, the electron transfer is expected to be from magnesium to aluminum in lower degree than magnesium to iron, as mentioned previously. However, in sample R-HbFe-C where the highest value of C/Fe was expected, this ratio turned out to be the lowest; thus, a large fraction of iron, as suggested by the Mg/Fe value, adopts the Fe^{2+} configuration.

3.3. Synthesis by simultaneous precipitation

The comparison of the X-ray diffraction patterns of samples prepared by simultaneous precipitation shows that in all cases well crystallized hydroxaluminates are obtained, Fig. S3. The 006 diffraction peak is broad and asymmetrical with the broader part located at small angles, showing some effort between the layers which must be due to a turbostratic arrangement.

Fig. 4 compares the morphology of sample S-HbAl-MW with the corresponding S-HbFe-MW. The sample containing aluminum presents a surface covered by flakes whose edges form an open network. The size of those flakes is ca. 50 nm. Instead, in the iron containing sample the surface is mainly polished. Samples S-HbAl-C and S-HbFe-C reproduce the features of the already mentioned aluminum microwaved preparation, Fig. S4.

The presence of hemoglobin is shown by the amount of carbon determined by EDS spectroscopy and FTIR spectra, Table 2 and Fig. S5. The atomic ratios $\text{Mg}/(\text{Al or Fe})$ are always lower than the nominal ratio of 4. The Mg/Al of the aluminum containing samples is ca. 3.15 and the C/Al ratio 20 and 12. Hence the amount of retained hemoglobin cannot be correlated to the Mg/Al ratio. It is interesting to note that, in the regenerated samples, the ratio Mg/Al was also close to 3.5, but, the retained hemoglobin per M^{3+} site was the same (C/Al around 6.5). The number of retaining hemoglobin sites, originated by the M^{3+} cations, is noticeably increased in simultaneous synthesis. Again, the aluminum containing samples retain more hemoglobin per site than the iron containing hydroxaluminates; such effect is enhanced in simultaneous precipitation. As previously suggested, in this type of synthesis, where the ratio Mg/Fe is anomalously low, a fraction of the Mg must be substituted by Fe^{2+} , showing that iron oxidation state is not only +3.

The specific surface areas of aluminum and hemoglobin containing samples, prepared through the simultaneous method, are ca. two-three times lower than the area of the corresponding reference samples, Table 1. The porosity for pores lower than 5 nm reproduces that of the Al-MW and Al-C samples. Note that the specific surface areas of samples S-HbAl-MW and S-HbAl-C are similar; hemoglobin, therefore, must be sorbed at the pore mouths occluding a fraction of them, as the peaks at 5 and 6 nm, present in samples Al-MW and Al-C, are not observed in the corresponding S-HbAl-MW and S-HbAl-C samples.

Again, if the specific surface areas of samples Fe-MW and S-HbFe-MW are compared the proportional factor is two. Comparing samples S-HbFe-C and Fe-C, the proportion is almost seven. This difference must be explained, not in terms of the amount of sorbed hemoglobin, but as a function of the variable oxidation state of iron. This result is in agreement with the proposition presented in the discussion of Table 2. This effect may be understood if the preparation methods are compared. The conventional procedure requires a long crystallization time; instead, a main advantage of the microwave irradiation method is the fast formation of the structure avoiding the reduction of iron ions. Of course, in aluminum

containing materials, the conventional preparation method cannot modify the initial aluminum oxidation state which is always three. Pore size distributions in both series reproduce the corresponding curves of the reference materials. Even if the second maxima in iron containing samples may shift, as the distributions are very broad in the range 10–50 nm, the modification of the peak location is not significant.

3.4. Synthesis by adsorption

The X-ray diffraction patterns of the aluminum containing samples, A-HbAl-MW and A-HbAl-C, correspond to well crystallized hydroxaluminates; no other compounds were identified. The A-HbFe-MW and A-HbFe-C diffractograms are less defined as the amount of background is much higher; thus, poor ordered compounds may be present as in some of the previous preparations, Fig. S6. Nevertheless, as these samples exhibit very low Mg/Fe and C/Fe ratios, we could also assume that the LDH amount is low, though Fe^{2+} was present in the layers.

The infrared spectra, Fig. S7, of the samples prepared through the adsorption method reproduce those already reported for simultaneously prepared samples. In all cases hemoglobin bands are superimposed to those of hydroxaluminates.

The SEM images of the aluminum containing samples correspond to a rather dense material with an apparently layered morphology, where some globular entities are evident, 50–100 nm in the microwaved sample, Fig. 5. In the conventionally prepared sample the size of the observed flakes is 100–300 nm. Instead, in the iron containing hybrids, Fig. S8, the arrangement of plates is house of cards like. In the microwave irradiated sample, the plates are smaller and, thus, the corresponding spaces among the plates are ca. 50 nm; instead in the conventionally prepared hydroxaluminates they are 100 nm.

From the EDS spectra, the atomic ratios were obtained, Table 2. The samples were rather homogeneous as the punctual analyses reproduced the results obtained for areas ca. $250 \mu\text{m}^2$. This preparation (adsorption method) provides an Mg/Al atomic ratio of 3.5, which is 0.5 lower than the nominal value. The amount of carbon per aluminum atom reaches 18.6 and 19.4 for A-HbAl-MW and A-HbAl-C samples, respectively. These values are close to that of the S-HbAl-MW sample (20.0), which is the highest value already obtained. This last sample had a 3.1 Mg/Al ratio, i.e. a value similar to the 3.5 obtained in the materials synthesized by adsorption. Hence, the amount of hemoglobin (proportional to C/Al ratio) is mostly determined by the aluminum content.

The ratios Mg/Fe and C/Fe are similar in the two iron containing samples. No differences can be attributed to the effect of microwave irradiation. The values are both very low indicating that a large amount of Fe^{3+} is reduced to Fe^{2+} , otherwise the hydroxaluminates structure could not be formed. Consequently the value C/Fe is very low.

The specific surface areas and the pore sizes distribution of aluminum containing hybrids, prepared through the adsorption method, Table 1, are similar to those of the corresponding samples prepared by the simultaneous method, indicating that hemoglobin is adsorbed on surface, blocking some pores. Instead, iron containing samples can be clearly distinguished by their specific surface areas, which in sample A-HbFe-MW is $46.4 \text{ m}^2/\text{g}$ and in A-HbFe-C $89.2 \text{ m}^2/\text{g}$, maintaining a pore size distribution whose main peaks are found at 3 and 8 nm. It seems that hemoglobin is an adequate agent to disperse hydroxaluminates particles creating nanometrical inter-granular cavities which constitute the main contribution to the specific surface area. This interpretation is in agreement with the micrographs discussed previously. The difference between the values measured for A-HbFe-MW and A-HbFe-C reveal that the A-HbFe-MW pores are more occluded than those of A-HbFe-C. Microwave irradiation disperses more the hemoglobin

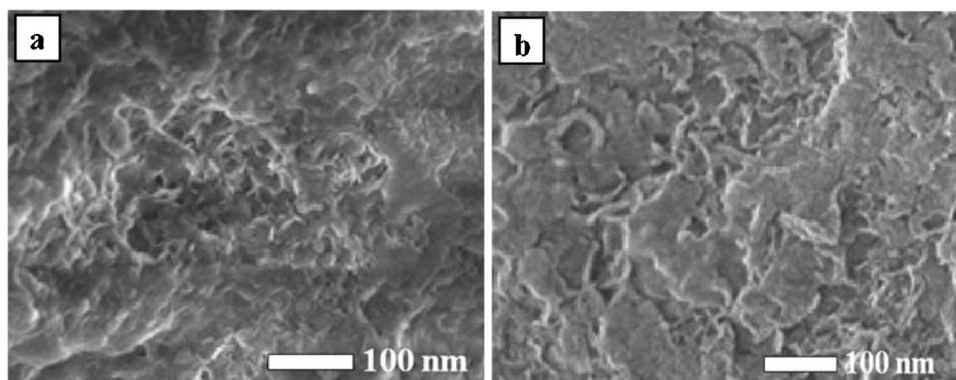


Fig. 4. SEM images of the samples prepared by microwave irradiation: (a) S-HbAl-MW and (b) S-HbFe-MW, both at a magnification of 50,000 x.

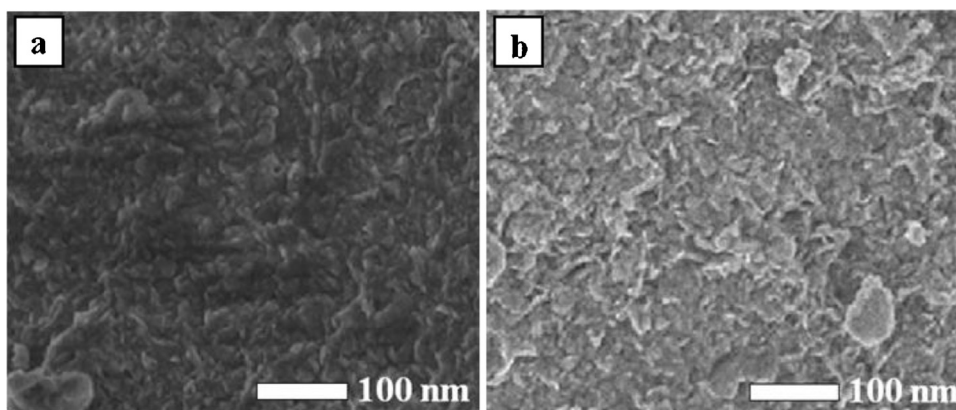


Fig. 5. SEM images of the aluminum containing samples: (a) A-HbAl-MW and (b) A-HbAl-C, both at a magnification of 50,000 x.

which can access in a higher number to hydrotalcite particles occluding them. EDS analysis shows, indeed, that A-HbFe-MW retains more hemoglobin per aluminum atom than A-HbFe-C sample.

4. Discussion

Our results may be summarized as follows. The reference sample features are most different depending on the M^{3+} cation. Iron promotes an open structure whose morphology reminds desert flowers. Instead, aluminum is characterized by smooth surfaces and low specific surface areas. Hemoglobin can be incorporated to those solids through three procedures: structure regeneration, simultaneous precipitation and adsorption.

In the regeneration process hemoglobin does not seem to participate in the hydrotalcite reconstruction process, only the sample R-HbFe-MW presents a significant amount of non-crystalline compound which can be interpreted as a poorly ordered hydrotalcite. Through simultaneous precipitation process, only the sample S-HbFe-MW surface seems to be polished; otherwise the other sample surfaces are covered by flakes whose edges form an open network. All specific surface areas are lower than $13 \text{ m}^2/\text{g}$. The adsorption synthesis method provided hybrids whose surface was smooth and dense if they included aluminum. Poorly ordered hydrotalcites are only observed in iron containing materials whose specific surface areas were found to be as high as *ca.* $90 \text{ m}^2/\text{g}$.

Therefore, composition and synthesis procedure, either in presence or not of microwave irradiation, determine the structure and morphology of the obtained solids. The purpose of our work was to prepare hybrids constituted by hemoglobin and hydrotalcite con-

taining aluminum or iron. Comparing the two groups of samples it is clear that aluminum, as it has only one oxidation state, induces well crystallized hybrid hydrotalcites where hemoglobin does not interact strongly with the support, mainly if they are prepared by the regeneration process. Hemoglobin must be retained by the charge deficiency present in pore mouths inhibiting the pore access. Specific surface area of these ordered solids is low. Hemoglobin may play the role of a template, altering the natural order of layered arrangement of hydrotalcites. In a previous work [19], it was found that the interactions between the template and the hydrotalcite precursors determine the hydrotalcite morphology and texture. The comparison among the hydrotalcites prepared using various organic fibrillar templates (hair, cotton or nylon) has shown that it depends on hydrophobicity, as well as on roughness and surface curvature.

Such effects are much more pronounced in iron containing materials [22]. Through the regeneration process the resulting hybrids are poorly ordered and comparable to those obtained adsorbing hemoglobin. In both cases, a non-negligible fraction of Mg cations must be substituted by Fe^{2+} , showing that iron oxidation state is not only +3. Iron definitively has to maintain the two oxidations states as it happens with cobalt hydrotalcites [23].

The following speculative mechanism can be advanced. Hemoglobin iron, in presence of carbon dioxide, is oxidized, *i.e.*, electrons are transferred towards this protein to the hydrotalcite, whose carbonates play a similar role as CO_2 . This reduction induces the formation of Fe^{2+} which, then, is structurally included in the hydrotalcite. The interaction between hemoglobin and Fe-hydrotalcite can be said to be strong, as it concerns electron transfers. Those characteristics suggest that these hybrids may

simulate photosynthesis as a heterogeneous process, Fig. S9. Such is not the case with aluminum containing hybrids, as aluminum has only one oxidation state. In this case the interaction is weak.

5. Conclusion

Mg–Al and Mg–Fe hydrotalcites presented very different behaviors in hemoglobin retention. Hemoglobin was strongly retained in iron containing hydrotalcites whereas aluminum hydrotalcites maintained their structure and were weakly bonded to hemoglobin. The interaction is favored by iron which promotes an open structure; the corresponding morphology reminds desert flowers. Instead, aluminum is characterized by smooth surfaces and low specific surface areas.

Hemoglobin is a protein with a very low charge density. Hemoglobin was retained in hydrotalcites whose electronegativity value was modified through the introduction of Al or Fe. Indeed, the electron transfer is from magnesium to aluminum in lower degree than from magnesium to iron.

Even in the regeneration process, which is the less favorable to interaction between the inorganic and the organic phases, the iron containing sample prepared in presence of microwaves presents a significant amount of non-crystalline compound, which can be interpreted as a poorly ordered hydrotalcite, only attributable to a strong interaction. The adsorption technique reveals that the contact between hemoglobin and hydrotalcite is favored by a high specific surface area and that it proceeds from the outer surface to the inner section of the particles. With adsorption time, hemoglobin manages to delaminate hydrotalcite lamellae arrangement.

The obtained materials should be highly suitable to retain CO as well as CO₂. The CO adsorbed on the composite may be oxidized in a second step. Most probably the resulting CO₂ will remain anchored on the solid. Such process should mimic the biological mechanisms observed in nature. These speculations will be the matter of a second work. As hemoglobin is a waste material in the meat process and hydrotalcites are economical, the proposed composite should be industrially affordable.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.cattod.2015.11.022>.

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