Short Communication

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Protein template effect on hydrotalcite morphology

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Layered double hydroxides (LDHs), hydrotalcite-like compounds, or hydrotalcites are porous materials, which may be used in catalysis, biomedicine, or adsorption as they may work as anion exchangers or drug deliverers. The morphology of hydrotalcites determines their use: in catalysis or in adsorption, reagents and gases have to reach as much surface as possible; in biomedicine, drugs have to be encapsulated and delivered later on. In this work, we synthesize hydrotalcites in presence of protein templates (egg white and yolk) and under two crystallization conditions (conventional and microwave). The obtained materials are hydrotalcites whose morphologies correspond to platelets organized as sand roses. Depending on the template and the preparation procedure, the amount of platelets can be increased. This feature is explained through the template effect of egg components. The resulting materials are promising adsorbers. Copyright © 2010 John Wiley & Sons, Ltd.

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Keywords: layered double hydroxide; microwave irradiation; white egg; yolk; albumin

INTRODUCTION

Layered double hydroxides (LDHs), hydrotalcite-like compounds, or hydrotalcites are porous materials.[1–3] They are anionic clays as they have positively charged layers due to a partial substitution of a divalent metal by a trivalent one. The charge is compensated by anions and water molecules intercalated between the layers. These materials can be represented by the general formula $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2](A^{m-})_{x/m}.nH_2O$ where M^{2+} and M^{3+} are the di- and tri-valent metals, respectively, and A^{m-} is a compensating anion.[4,5] Although it is possible to obtain various LDHs modifying the composition and the synthesis process, the most common LDH is $Mg_6Al_2(OH)_{16}CO_3.4H_2O$. The usual synthesis procedure is co-precipitation of the cationic metals in a basic medium (sodium hydroxide), followed by a conventional hydrothermal treatment.[6,7]

Activated Mg/Al hydrotalcites are efficient base catalysts in aldol condensation reactions. Their performance has been increased eight times just varying the morphology.[8,9] Nanometrical platelets, obtained through the reconstructive method under high stirring or ultrasound irradiation, are the most efficient.

Still, some authors have recently reported on the use of templates to modify morphology and hence texture.[10,11] Those inducing agents are alcohol or acetone molecules; in this case a vesicular shape is obtained.

No special attention has been focused on the effect of natural proteins on the morphology of these solids. Albumin, for instance, whose molecular weight is *ca*. 68,500, has a large size around 0.5 μm and a rather elongated elliptical shape.[12] It should act, indeed, as a macromolecular polymer.

In this work, we study the synthesis of hidrotalcite-like compounds in presence of egg white and egg yolk, either in the conventional way (plate heating) or employing microwave irradiation during the hydrothermal step.

EXPERIMENTAL

Hydrotalcite synthesis

Mg/Al-hydrotalcite samples were synthesized from a $Mg(NO_3)_2.6H_2O$ and $Al(NO_3)_3.9H_2O$ (both from Aldrich) 3.3 M water solution, a 2 M NaOH (Baker) aqueous solution and three different templates. Organic templates present in hen egg were used (yolk, white and white + yolk) as follows: over a Becker recipient containing 10 ml of template and 25 ml of a NaOH solution, with stirring, the Mg-Al solution (110 ml) simultaneously was added dropwise with the remaining NaOH solution, adjusting the flow of both solutions to maintain a constant pH of 11. The solution amounts correspond to a molar ratio Mg/Al of 3. The white + yolk proportion corresponds to a 65 and 35 wt%, respectively. After precipitation, the resulting gels were hydrothermally treated in a microwave autoclave (MIC-I, Sistemas y Equipos de Vidrio S.A. de C.V.) for 10 min at 80°C, operating at 2.45 GHz and a power of 200 W. The solids were recovered by decantation and washed several times with distilled water until the residual solution reached a pH value of 9.5 (+0.3). The solids

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were, then, dried in an oven at 70°C overnight. The samples were labeled W-MW, Y-MW, and YW-MW depending on the used template: white, yolk, or white + yolk, respectively. Other samples were prepared following a similar procedure by using a conventional hydrothermal treatment instead of employing microwave irradiation. The mixture obtained after solutions precipitation was hydrothermally treated in an open reactor stirred for 24 hr at room temperature (25°C): samples W-C, Y-C and YW-C for white, yolk, and white + yolk template, respectively. Lastly, a reference sample, R-MW, was prepared in the same way, without template and under microwave irradiation.

Characterization

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 Kα radiation.

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.

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 with the BJH method applied to the desorption branch. The samples were pretreated at 200°C for 10 hr at high vacuum.

Scanning electron microscopy

A scanning electron microscope LEICA, Stereoscan 440 was used. The samples were previously covered with gold to avoid charge problems.

RESULTS

X-ray diffraction

The template effect on the structure of hydrotalcites was studied by comparing samples synthesized by two different hydrothermal treatments: microwave or conventional. X-ray diffraction patterns of all the samples are presented in Fig. 1, they all correspond to a hydrotalcite (JCPDS 22-0700) comparable to the reference sample (R-MW). No other crystalline compounds were identified.

For the samples treated with microwave irradiation the 00l peaks are very intense, even when compared with those of the reference sample, R-MW, showing that hydrotalcites are well crystallized. In these materials the peaks 110 and 130, in the 2θ interval 58–63°, are well resolved showing a good long-range order in the layers, i.e. in *a* and *b* directions.

Instead, the samples prepared by the conventional way, i.e. without microwave irradiation, show less intense diffraction peaks indicating not well stacked layers or smaller particle sizes. In all cases, the interlayer distance, determined from the 003 peak

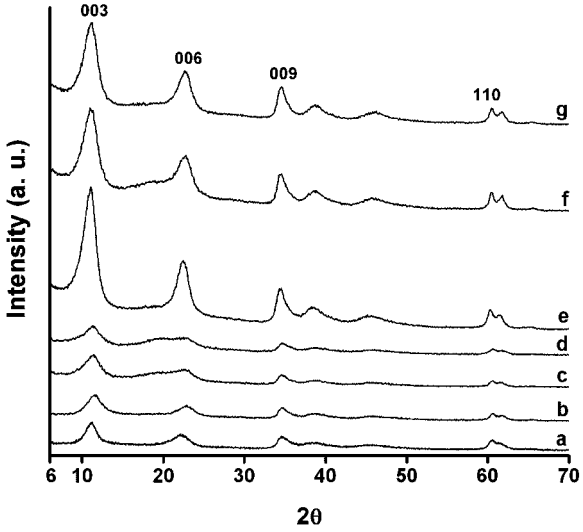

Figure 1. X-ray diffraction patterns of the samples: (a) R-MW, (b) W-C, (c) Y-C, (d) YW-C, (e) W-MW, (f) Y-MW, and (g) YW-MW.

position, was *ca.* 8.4 Å, indicating that the interlayer anions must be nitrates and some carbonates formed from the CO_2 absorption during the precipitation step of the synthesis.[13,14]

These results show that, on the one hand, the combination of template with microwave irradiation promotes crystal growth and, on the other, the templates do not alter the interlayer distances, i.e. they are not intercalated.

FTIR spectroscopy

The comparison of the white and yolk spectra shows that they can be distinguished through this method; yolk presents an intense band at 1749 cm^{-1} which is not present in white. The hydrotalcite prepared without template shows the usual bands at 1390 cm^{-1} attributed to inter-layered nitrates and carbonates.[14,15] The band at 3447 cm^{-1} is attributed to the OH structural groups and the band at 1651 cm^{-1} to the water hydroxyls.[4]

The sample prepared in presence of white is intercalated with nitrates and carbonates as the band at 1385 cm^{-1} is clearly resolved. The broad structural OH band is, of course, also observed. A small new band at 1545 cm^{-1} is interpreted as due to the vibration of N–H and C–H groups present in the protein.[16] Hence, the desert rose like nanoparticles encapsulate the albumin macromolecules, which are located among the hydrotalcite assembled layers.[17]

The yolk prepared sample presents similar features, indeed, the bands corresponding to hydrotalcite and yolk are present. The peak appearing at 1750 cm^{-1} is due to C=O bonds. The other peaks, 1670 and 1567 cm^{-1}, are due to N–H and C–H groups as in the pure yolk spectrum.

The spectrum of the white + yolk preparation is intermediate, independently of the crystallization method (microwave irradiation or not). Still, the relative intensity of the bands varies showing that with the conventional preparation method the egg species are better preserved.

Morphology

All samples prepared with template and in presence of microwave irradiation consist of large irregular and compact chunks (3–4 μm)

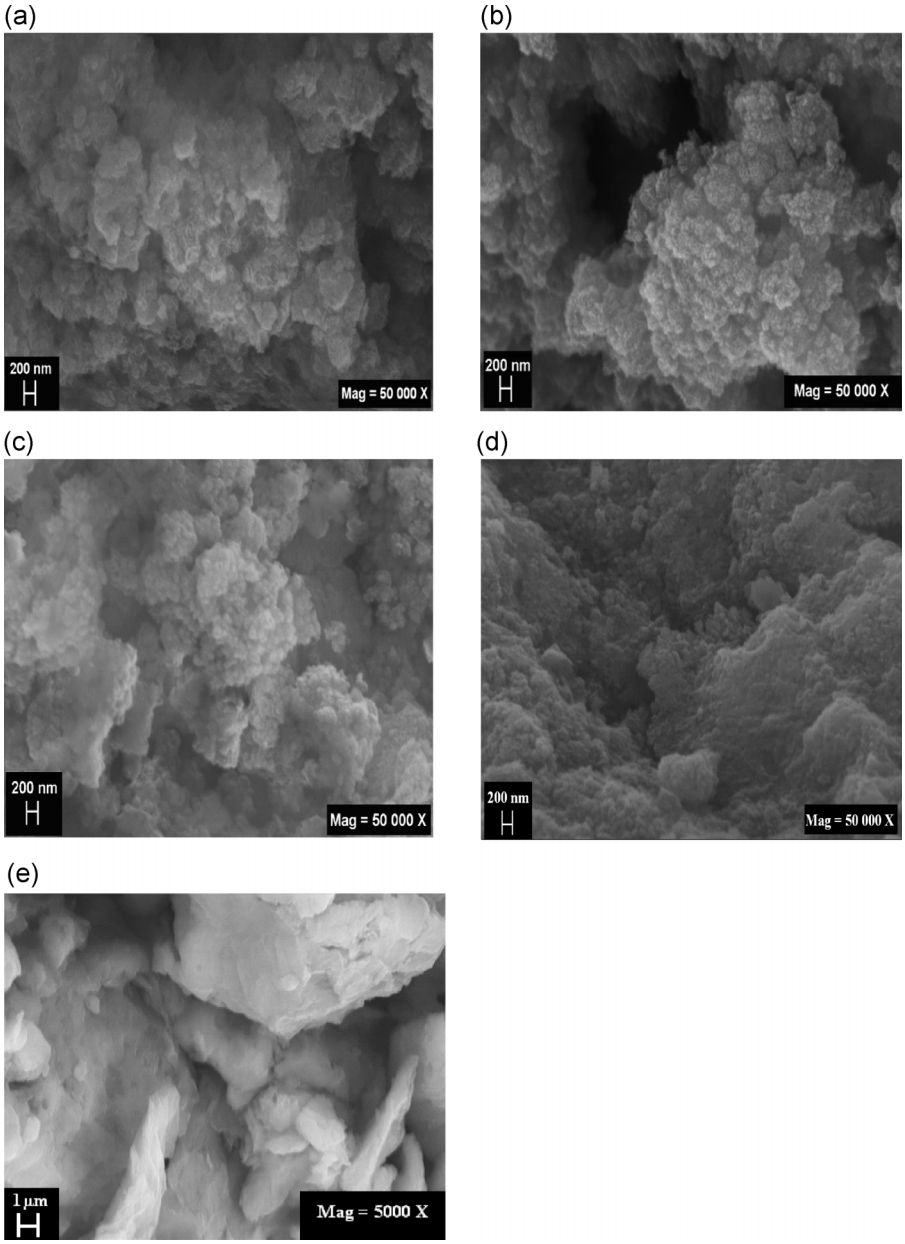

Figure 2. Scanning electron micrographs of the samples: (a) W-MW, (b)YW-MW, (c) W-C, (d) YW-C, and (e) R-MW.

constituted by smaller spherical particles (*ca*. 100 nm), as shown by SEM in Fig. 2. In the images the cauliflower-like particles appear to be stuck together, separated by large pores whose size is around 1–2 μm. Instead, the micrograph of the reference sample, R-MW, prepared without template, shows that the chunks are bigger than 3 μm and present a smooth surface. If egg white is used as template, small particles are formed. They are constituted by very small layers organized as sand roses. Such morphology has been reported for the high base supersaturation conditions imposed by co-precipitation method.[18] The YW-MW sample is denser, the very small globular particles are constituted again by sand roses in such number that they cannot grow as large as those observed in the previous sample. It seems, then, that the presence of yolk promotes the formation of a higher number of nucleation centers.

If the samples are not microwave irradiated, the hydrotalcite prepared in presence of yolk turned out to be rather different to the corresponding one prepared in presence of microwaves. The particles are smaller (less than 50 nm) and cover very large chunks, which seem to correspond to a conventional hydrotalcite where a macroscopic layered structure can be observed. It has to be emphasized that the reference sample (R-MW) has a stratified morphology and very large particles, Fig. 2. Instead, if the sample is prepared in presence of white, the resulting compound is heterogeneous: small sand roses are observed together with layered and smooth particles, typical of hydrotalcite. Most probably albumin is located in the sand roses as suggested by infrared spectroscopy.

Texture

With microwave irradiation the specific surface areas are comparable as they are 90 (sample W-MW) and 110 m^2/g (Y-MW and YW-MW samples). Instead, the YW-C sample has a

surface area of 20 m^2/g only, which is five times lower. Yolk and white containing materials (Y-C and W-C) present 56 and 164 m^2/g, respectively. None of these values reproduce the surface area of the reference sample (86 m^2/g).

The corresponding pore size distributions are all unimodal and close to 30 Å for samples prepared conventionally. In the microwave-irradiated samples, a broad peak with a maximum at a radius of 45 Å is also observed.

Such results may be correlated with those obtained by SEM. The largest area (164 m^2/g) was obtained in W-C sample where sand roses and smooth hydrotalcite were observed. In this sample, all surface is reached by nitrogen molecules. Instead, in sample YW-C, which is also constituted by hydrotalcite chunks covered by small particles, the specific surface area is 20 m^2/g. In this sample, then, the access to the surface area of hydrotalcite is blocked either by the small particles or by protein residues adsorbed at the layer mouths. It is interesting to note that the two microwaved preparations present a rather similar areas as both samples are constituted by sand roses whose surface area can be reached by gases; this surface area is higher than the one found in the reference sample.

DISCUSSION

Summarizing our results, it was shown by XRD that all samples are hydrotalcite. No other crystalline compounds were present. The only differences are morphological. Indeed, the scanning electron micrographs of the microwave-irradiated samples are similar. The samples prepared with yolk, white, or white + yolk seem to be an assembly of very small sand roses. In the conventional preparation, yolk prepared material reproduces the previous features whereas the presence of white and white + yolk generate more heterogeneous materials constituted by platelets and small aggregates. The three microwaved samples presented a bimodal pore size distribution, but the other three distributions were monomodal. As far as the surface areas are concerned, again the microwaved compounds are similar. With this technique a clear difference is detected between the yolk and the white or white + yolk materials.

In the reference sample, only a stratified morphology and a surface area equal to 86 m^2/g were determined. The pore size distribution was monomodal with a maximum at 30 Å. Therefore, the template effect of yolk, white or white + yolk consists, in presence or not of microwaves, in the formation of nanometric platelets organized as sand roses. They may be assembled to constitute large agglomerates, then, the surface area may vary. Most probably the organic macromolecules are retained in the interparticle assemblies.

In previous works, we have studied the microwave effect on hydrotalcite synthesis without template.[13,14] Microwave irradiation diminishes synthesis time and alters nucleation mechanisms as more nuclei are simultaneously formed, then the resulting particles are smaller. Still, they are always flat. As stated by Benito *et al.*[19] growing of the particles under conventional heating takes place following the temperature gradients by dissolution and precipitation of the particles, leading to the formation of larger particles (Ostwald ripening), while under microwave treatment the reduction of temperature gradients might lead to a simultaneous dissolution which involves a simultaneous nucleation, resulting in a higher number of smaller particles.

However, other authors have found that a vesicle shape may be induced through alcohol or acetone templates.[10,11] Urea has been also tested,[18,20] these studies have been carried out without microwave irradiation. Our results have shown that microwave irradiation, again, in presence of template promotes a different crystallization mechanism. Note that albumin is not decomposed in our working conditions.[21]

Template has a clear effect on the resulting samples as platelets are formed. Only in presence of microwave irradiation the sample turns out to be fully constituted by platelets organized as sand roses. Instead, if the samples are synthesized in the conventional way stratified layers of hydrotalcite with sand roses are present.

For low pH values the charged groups present in proteins are protonated and the net charge is positive. Whereas, if the pH value is high, as in our experiments, those charged groups turn out to be negative, they attract then, the positively charged hydrotalcite layers. In presence of microwave irradiation the crystallization is very fast and hence proteins are not able to denaturalize. Therefore, hydrotalcite crystallizes in presence of a charged ellipsoidal template[12] which separates the platelets. In the conventional procedure, where the stirring time was as long as 24 hr, the protein can denaturalize to form linear chains, which, although they are charged, favor a lamellar morphology. Note that they are so large that they do not fit between the layers. Infrared spectra confirm such propositions as some protein residues are always found even after thoroughly washing, showing a strong interaction. Those adhered proteins explain the various surface area values. The features of the prepared materials, then, fulfill the requirements to retain negatively charged molecules. The platelet morphology should favor a rapid exchange with nucleosides and DNA to develop gene delivery carriers.[22] Furthermore, the delivery of a full gene through the use of nanobiohybrids based on hydrotalcite has been already reported.[23] In those applications the morphology and platelet size are crucial.

CONCLUSION

The treatments during the hydrothermal step (conventional or microwave irradiation) determine the morphology and texture of the obtained hydrotalcite prepared in presence of egg proteins. The obtained materials are hydrotalcite whose morphology corresponds to platelets organized as sand roses. Depending on the template and the preparation procedure the amount of platelets can be increased. Our results open a new controlled way to produce, on request, nanometrical platelets or conventional stacked layers, just changing the egg template agent and/or the energy flux. Note that the area values for the protein templated materials in presence of microwave irradiation are fairly high.

The advantage of this new procedure is the time required to prepare those original samples. Last but not least, the used templates are economical and easily available.

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