A new in situ enhancement of the hydroxyapatite surface by Tyramine:
Preparation and interfacial properties

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ABSTRACT

The grafting of biogenic monoamine can provide an effective route to modify the surface properties of apatite materials. This study presents the modification of the hydroxyapatite surface by Tyramine prepared by hydrothermal reaction. The nanoparticles of Tyramine grafted hydroxyapatite were characterized by x-ray diffraction, infrared spectroscopy, solid-state NMR spectroscopy, thermal analysis, TEM microscopy, and measurements of the specific surface area (S_{BET}). In situ grafting of Tyramine onto the HAp surface reduces crystal growth through the formation of organic-inorganic hybrid nanoparticles, thereby developing a multifunctional surface, thus providing a good compatibility with surface interaction for cell-modified HAp. To better illustrate the surface properties of the prepared biomaterials, the interaction of methyl-orange with the modified HAp was studied using a modified method developed within the framework of this work. Effect of the grafting rate on the adsorption performance was examined. The adsorption kinetics were well described by a second order model, which is remarkably influenced by the Tyramine grafting rate.

Keywords. Hydroxyapatite, Tyramine, Grafting, Surface reactions

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1. Introduction

Hydroxyapatite Ca_{10}(PO_4)_{6}(OH)_2 (HAp) is a naturally occurring mineral widely used in development of bioceramics, environmental sorbents and heterogeneous catalysis [1-4]. Its reactivity depends on different parameters such as particles size, porosity and crystallinity [5-7]. In recent years, several studies have shown that the addition of a wide range of additives, such as silane [8] and titanate coupling agents [9], biomacromolecules [10] and polymers [11] can control the nucleation of hydroxyapatite and modify its surface properties. In addition to these, other studies have ascertained the grafting effect of organic molecules on the crystallization of hydroxyapatite. Among them, amino additives have important applications in the process of biomineralisation, the most important for controlling the nucleation of biomaterials. This approach controls and promotes the active sites of the HAp surface, which will condition the properties of the good adsorbent. Applications of grafted hydroxyapatites are based on their physicochemical properties, in particular the reactivity of their surface. The modified hydroxyapatites are well known for their surface and ionic exchange properties with various organic and inorganic substances [12-13]. Hydroxyapatite surfaces have mainly been changed by grafting organic or biological species involving several intermediate steps of the reaction [14-15]. In addition, it is possible to use biologically active molecules such as amino acids for their importance in biomedicine [16]. These approaches not only allow the homogeneous grafting of the active organic functions over the whole material volume, but the amino acids can affect the crystalline growth of calcium phosphate particles and create new active sites for the binding of biological or others species [17-18]. As a result, it appears that these surface modifiers are the most promising owing to their high affinity for Ca^{2+} ions, which mainly constitute hydroxyapatite or natural bone. The modified hydroxyapatites are commonly used in the field of medicine and are considered as a promising alternative to better promote the ion exchange between biologic fluid and the implant, which facilitates the binding of certain
biological species or undesirable species. As health and environmental applications, the pure HAp was widely used to remove azo dyes such as methyl orange, but its retention capacity was very limited. So, its grafting by Tyramine as a chelating agent can improve its surface properties and subsequently improve the retention of coloring species. Tyramine is a biogenic monoamine produced by amino acid decarboxylation and exists in a variety of foods (such as meat, aquatic products, fermented and other products) [19-20]. It is characterized by a bifunctional character of the substrates (phenol and amine). Its structural and electronic properties (electron-rich cavity formed between the aromatic rings) make its potential hosts that are useful for selective sensors of electrophilic species, such as metal cations, leading to the formation of macrocyclization matrices in the aqueous solution. Some reports have also shown the good chemical affinity of tyramine with clay matrices for the production of nanocomposites [21-23]. In this work, we examine the affinity of hydroxyapatite towards amino acids namely the Tyramine prepared by using a hydrothermal method. Various Tyramine-grafted HAp materials are prepared and characterized. The surface properties of these organoapatites are tested by the adsorption of methyl orange, C_{14}H_{14}N_{3}NaO_{3}S (so-called MO), which is frequently used in titration because of its clear and distinct color variance at different pH values. Among the dye removal techniques in aqueous solutions, adsorption is the most used because it is effective and used successfully at large scale in the industry using suitable adsorbents [24-26]. Kinetics of methyl-orange (MO) uptake by the surface of Tyramine-modified hydroxyapatites were investigated by evoking the effect of the Tyramine amount incorporated into the HAp structure. The main objective of this study is to improve the efficiency of the process allowing the retention of the maximum elements while seeking to optimize the conditions of synthesis and operation, which mainly depend on chemical reactions and physical phenomena.

2. Experimental

2.1. Materials
The powders were prepared using Calcium nitrate [(Ca(NO$_3$)$_2$ 4H$_2$O), Riedel-de Haen 99%], ammonium dihydrogen phosphate [(NH$_4$)H$_2$(PO$_4$), Merck 99%], ammonia (Prolabo, $d = 0.89$; 28% aqueous solution) and Tyramine [C$_{8}$H$_{11}$NO, Aldrich > 98%]. All materials were used as received without further purification. As shown in figure 1, tyramine is an organic chemical compound of monoamine type which belongs to the family of phenylethylamine.

![Fig.1. Formula C$_8$H$_{11}$NO, (4-(2-aminoethyl) phenol), Molar mass = 137.179 g.mol$^{-1}$, XLogP3 = 1.1, Water solubility = 10.4 g/L at 15 °C, pK$_{a1}$ = 9.1 and pK$_{a2}$ = 10.9](image)

### 2.2. Powders synthesis

The pure HAp was prepared by mixing 10 mmol of calcium nitrate (Ca(NO$_3$)$_2$ 4H$_2$O) with 6 mmol of ammonium dihydrogenate phosphate (NH$_4$)$_2$HPO$_4$ and adjusted at pH 10 by addition of ammonium hydroxide. The solution was maintained under stirring using N$_2$ atmosphere at room temperature. The mixture was introduced in an autoclave under hydrothermal conditions at 100°C for 12 hours, followed by filtration, washing with bidistilled water and drying at 100°C overnight. The hybrid Tyramine-HAp materials were prepared according to the same experimental protocol by adding different Tyramine amounts to the calcium solution. The hybrid samples nTyr-HAp is defined with different hybrid samples, where n is the tyramine/HAp molar ratio (n = 0, 2, 4 and 6).

### 2.3. Materials and methods

X-ray diffraction analysis was carried out by means of a X'Pert Pro Panalytical X-Pert diffractometer using Cu-K$_{a}$ radiation ($\lambda = 1.5418$ Å), equipped with an X'Celerator solid detector and a Ni filter. The experimental patterns were compared to standards compiled by the Joint Committee on Powder Diffraction and Standards (JCPDS cards) using the X'Pert High-
Score Plus software. The lattice parameters (a, c) were determined by Rietveld refinement of the XRD data using the Fullprof program. The infrared spectra were obtained using a spectrum Two 104462 IR spectrophotometer equipped with a diamond/ZnSe ATR crystal. Elementary analysis was determined using ICP-OES on a Horiba Jobin Yvon. The thermal properties were determined using a SETARAM SETSYS. High Resolution Transmission Electron Microscopy (HR-TEM) observations were performed on a JEOL JEM 2010 transmission electron microscope equipped with a LaB₆ filament and operating at 200 kV. Specimens were prepared by depositing a drop of these aqueous suspension materials onto 300 mesh Cu grid coated with a lacey carbon film. Prior to deposition, samples were sonicated for 5 min. Solid-state MAS NMR spectra were recorded at 162 MHz on a Bruker Avance 400 spectrometer (4 mm diameter rotor, spinning rate up to 12 KHz). Specific surface areas were determined by multi-point N₂ gas sorption experiments at 77 K using a Micromeritics ASAP 2010 instrument according to the Brunauer-Emmett-Teller (BET) method using adsorption data in the 0.05 to 0.25 relative pressure range. Pore size distribution was determined using the Barret-Joyner-Halenda (BJH) approximation in the mesoporous range.

2.4. Adsorption kinetics studies

The kinetic adsorption of methyl orange (MO) was carried out by mixing 250 mg of nTyr-HAp with 100 ml of aqueous solution of MO of 20 mg L⁻¹ initially adjusted at pH 4. Samples were centrifuged and the residual MO concentration was determined by UV-visible spectroscopy at λ= 464 nm. The amount of sorbed MO, qₜ (mg g⁻¹), at time t was calculated as follows:

\[ q_\text{t} = (C_0 - C_\text{t}) \cdot V / m \]

where \( C_0 \) and \( C_\text{t} \) are the initial MO concentration (in mg L⁻¹) in the liquid phase initially and at any time \( t \) respectively, “m” is the mass (g) of the solid in the solution, and \( V \) is the solution volume (L). In order to examine the mechanism of the adsorption process (mass transfer, chemical reaction); we examined kinetic models to analyze our experimental results. Lagergren [27] proposed a kinetic model of the pseudo first order expressed by the following equation:

\[ \log(q_e - q_\text{t}) = \log q_e \cdot k_1 \cdot t / 2.303 \]

where \( k_1 \) and \( q_e \) are the rate constant for...
pseudo first order and the maximum adsorption capacity respectively. To get as close as possible to the real reaction mechanism, Ho and Mc Kay [28] have opted instead for a kinetic model of order 2. They suggest the existence of chemisorption. This model is described by 
\[ t/q = 1/(k_2 q_{e,2}^2) + t/q_{e,2}, \]
where \( k_2 \) and \( q_{e,2} \) are the rate constant for pseudo second order and the maximum adsorption capacity respectively.

3. Results and discussion

3.1. Structural properties

X-ray powder diffraction analysis of the ungrafted and tyramine-grafted HAp materials are shown in Fig. 2. All samples exhibit diffraction peaks of the apatite structure with a poor crystallinity as indicated by the broad half-peak width. A loss of crystallinity with increasing Tyramine amount is observed. The crystallite size as estimated by the Scherrer formula is given in Table 1. The significant reduction of crystallite size with increasing the tyramine content shows a preferential interaction of the tyramine with HAp faces parallel to the c-axis. Results are in a good agreement with those described elsewhere using glutamic and aspartic acids [18]. The lattice constant values \( a \) and \( c \) are also presented in Table 1. In increasing the tyramine amount in HAp, the a-axis parameter slowly increases from \( a=9.434 \) Å for \( n=2 \) to \( a=9.452 \) Å for \( n=6 \), while \( c \)-parameter remains constant at \( c=6.88 \) Å. This evolution confirms the preferential interaction between \( \text{Ca}^{2+} \) ions and the amine -NH\(_2\) (Ca....NH\(_2\)) or hydroxyl (Ca.....O-Tyr).
Fig. 2: X-ray diffraction patterns of Tyramine-grafted hydroxyapatite materials

Table 1: Structure properties of HAp before and after functionalization.

<table>
<thead>
<tr>
<th>Samples</th>
<th>D_{002}/D_{310}^a</th>
<th>X_c^b (%)</th>
<th>a^c (Å)</th>
<th>c^c (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAp (n=0)</td>
<td>2.412</td>
<td>1.981</td>
<td>9.407(9)</td>
<td>6.886(1)</td>
</tr>
<tr>
<td>n=2</td>
<td>2.529</td>
<td>1.355</td>
<td>9.434(2)</td>
<td>6.882(3)</td>
</tr>
<tr>
<td>n=4</td>
<td>2.829</td>
<td>0.393</td>
<td>9.441(5)</td>
<td>6.881(9)</td>
</tr>
<tr>
<td>n=6</td>
<td>2.876</td>
<td>0.233</td>
<td>9.452(8)</td>
<td>6.878(4)</td>
</tr>
</tbody>
</table>

^aRatio of coherent lengths in the direction normal to (002) and (310). ^bCrystallinity, ^cUnit cell parameters.

The IR spectra recorded before and after surface modification of HAp by tyramine are shown in Fig.3, including the related PO_4 group vibration bands and new absorption bands after tyramine functionalized -HAp surface. The C-H stretching frequency is observed at 3000 cm^{-1} for the protonated tyramine. The symmetric C-H stretching vibration is obtained at 2970 cm^{-1}
and asymmetric C-H stretching vibration is obtained at 2937 cm$^{-1}$ in good agreement with those published elsewhere [18, 29-31]. The bands at 1413 and 1350 cm$^{-1}$ are attributed to aliphatic and aromatic C-C bond, respectively. On the other hand, the amine absorption band (N-H) is found at 3280 cm$^{-1}$ and C-N towards 1517 cm$^{-1}$.

![IR spectra of tyramine-grafted hydroxyapatite materials](image)

**Fig.3:** IR spectra of tyramine-grafted hydroxyapatite materials

To confirm furthermore tyramine grafting in the apatite structure, the solid-state NMR spectroscopy is a helpful adjunct to XRD and IR techniques. $^{31}$P NMR-MAS spectra of Tyr-modified HAp show a single intense isotropic signal at 2.8 ppm that confirms the presence of one crystallographic site of apatitic phosphorus ($\text{PO}_4^{3-}$) (Fig 4a). In addition, three $^1$H MAS-NMR signals are observed at 0 ppm, 1.35 ppm and 5.30 ppm attributed to the protons of hydroxyl OH, -CH$_2$ groups, and to the adsorbed H$_2$O molecules on the apatite surface, respectively (Fig.4b). The nTyr-HAp spectra also show an additional peak (1.35 ppm) not detected in the HAp spectrum, which represents signal from proton of -CH$_2$ groups of tyramine, added in matrix after HAp surface grafting. Intensity of this signal increases by increasing the
amount of tyramine. Therefore, this additional signal results from the modification of the surface by tyramine.

Fig. 4. Solid-state MAS-NMR spectra of grafted hydroxyapatite nTyr-HAp powders.

Thermal gravimetry (TG) was used to get the further stability properties of the hybrid materials during heat treatment compared to the pure hydroxyapatite. Two main thermal effects are observed in TG analysis (Fig. 5). A small mass loss is observed before 120°C assigned to water physical desorption, whereas the second is due to the decomposition of Tyramine molecules grafted into the apatite layer. The weight loss increases with increasing Tyramine content where a strong weight loss of 8.3wt% is found for 6Tyr-HAp in agreement with IR results. The chemical analysis of calcium, phosphorus and carbon is also summarized in Table 2. The molar ratio Ca/P of HAp powder is close to 1.68 which decreases for hybrid materials with a dramatic decrease for 6Tyr-HAp (Ca/P=1.61) related to the important grafting of tyramine in apatite structure. At higher tyramine content (6Tyr-HAp), the decrease in the Ca/P ratio can be explained by the fact that the apatite particles are terminated on the surface by the tyramine molecules causing perhaps a low loss of calcium. As shown in Table 2, the carbon content is
seen to increase with the amount of incorporated tyramine species and is in good agreement with TG results, suggesting a pronounced effect on HAp formation. Results confirm the interaction between tyramine and HAp surface (Tyramine....HAp) and predict the formation of new hybrid organoapatite materials.

![TG curves of pure and hybrid hydroxyapatites.](image)

**Fig. 5:** TG curves of pure and hybrid hydroxyapatites.

**Table 2:** Elementary chemical and TG and surface analyses of nTyr-HAp powders.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Ca/P</th>
<th>C (%)</th>
<th>TG (wt%)</th>
<th>$S_{BET}$ (m$^2$·g$^{-1}$)</th>
<th>$D_p$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAp</td>
<td>1.68</td>
<td>0.12±0.07</td>
<td>2.2±0.13</td>
<td>44±0.5</td>
<td>43</td>
</tr>
<tr>
<td>2Tyr-HAp</td>
<td>1.67</td>
<td>0.94±0.09</td>
<td>4.8±0.21</td>
<td>108±0.9</td>
<td>17</td>
</tr>
<tr>
<td>4Tyr-HAp</td>
<td>1.65</td>
<td>1.36±0.10</td>
<td>7.1±0.25</td>
<td>35±0.6</td>
<td>54</td>
</tr>
<tr>
<td>6Tyr-HAp</td>
<td>1.61</td>
<td>2.30±0.11</td>
<td>8.3±0.31</td>
<td>28±0.4</td>
<td>68</td>
</tr>
</tbody>
</table>

These hybrid materials were then investigated by transmission electron microscopy to check the homogeneity of the samples and, more particularly, to study the dispersion of Tyramine within the apatite network and to assess particle morphology and size. **Figure 6**
shows the TEM images of unmodified HAp and Tyr-modified HAp nanoparticles. nTyr-HAp samples consist of well-defined nanorod morphologies but with different lengths, i.e. between 50 and 120 nm. From TEM images, it can be understood that the amount of tyramine had an influence on the aspect ratio of the nanocrystal particles in the hybrid organoapatite. This result is in agreement with the increase of the $D_{002}/D_{310}$ ratio from XRD analysis.

![TEM images of nTyr-HAp (n=0, n=2, n=4 and n=6)](image)

**Fig. 6.** TEM images of nTyr-HAp (n=0, n=2, n=4 and n=6)

Nature of the interactions between the apatite surface and Tyramine depends on the pH of the precipitation reaction. Taking into consideration the fact that the grafting reaction is performed at pH 10, it can be considered that Tyramine in aqueous solution exists as various species where for higher-basicity amino NH$_2$ and/or hydroxyl OH can be complexed with Ca$^{2+}$ cations (Fig. 7). The formation of Ca$^{2+}$-NH$_2$(Tyr) complexes has well profound significance in biology [32]. The specific surface area of nTyr-HAp powders are gathered in Table 2. The largest area was obtained for 2Tyr-HAp (108 m$^2$g$^{-1}$) and when the Tyramine content incorporated in the
HAp matrix increases more than n=2, the area becomes smaller. The dramatic decrease in specific surface area after grafting is due to the excess of organic moieties occupying the pores. From Table 1, the pore diameter (Dp) in functionalized apatite is slightly larger than that of HAp except for 2Tyr-HAp where it has a low value. This is related to the tyramine content and the Ca2+ - Tyr electrostatic interactions.

![Fig. 7. Schematic grafting of tyramine on HAp surface.](image)

### 3.2. Tyramine-grafting ratio effects on methyl orange adsorption

The effect of contact time on the uptake of MO dye by nTyr-HAp materials is showed in Figure 8. By increasing the incubation time, the dye retention efficiency increases up to 20 min to reach equilibrium. After this time, the MO sorption varies slightly and equilibrium is reached. The grafting of a small amount of Tyramine (n=2) improves the removal of MO from the aqueous solution, but its further increase reduces the adsorption capacity. Results can be explained by the saturation of the sorbent surface by tyramine and are in good agreement with the S\textsubscript{BET} values and particle sizes. From an initial pH 4 of the aqueous orange methylene solution, the equilibrium pH of the MO/nTyr-HAp system is between pH 6-7 depending on the grafted apatite, but more important in the case of pure HAp (close to pH 8. This increase in
equilibrium pH is due to the adsorption process takes place in two stages, the first involving a strong interaction between the grafted apatite surface and MO molecules, causing the protonation phenomena and then the solution becomes more alkaline. It can therefore be supposed that different types of interactions between tyramine distributed onto Tyr-HAp surface and MO molecules. On the other hand, the pH value at the point of zero charge (pH_PZC) value is an important parameter influencing the mechanism of adsorption when the pH_PZC value is between 6.5 and 8.5 [33-35]. If the pH is greater than pH_PZC, the surface is negatively charged while for low pH values, the surface sites are positively charged. For this, the adsorption of MO on the grafted hydroxyapatites is governed by the electrostatic interactions between the surface of the positive sites and the negatively charged MO groups [36]. By increasing the pH, the surface becomes more negatively charged, which explains the decrease in adsorption by increasing the Tyramine content in HAp matrix. This result is more significant for 2Tyr-HAp sample, characterized by a high fixation of the MO dye linked to its good value of the specific surface and the presence of complexing amino groups as new active sites. To study the adsorption mechanism further, simulation of the kinetics data was carried out by using pseudo-first, and pseudo-second order. The adsorption kinetic parameters are reported in Table 3. Based on the correlation coefficients (R²) and the theoretical sorption capacities that coincide with the experimental values, the MO adsorption process is correctly described by the pseudo-second-order model, suggesting the possibility of chemisorption. This adsorption involves the exchange or sharing of electrons between dye and Tyr-HAp surface.
Various mechanisms are responsible for the fixation of dyes on the hydroxyapatite surface, but most of them are usually combined between surface complexation reactions and Van Der Waals interactions [37-38], but the large specific surface is a factor not to be excluded for the
adsorption of MO, as proven by the good adsorption performances of the 2Tyr-HAp sample which has the largest specific surface area. Figure 9 shows the three possible functions of methyl orange that can be attached to the surface of Tyr-HAp nanoparticles under acidic pH conditions.

Fig.9: Illustration of the adsorption mechanism of methyl orange on the Tyr-HAp hybrid hydroxyapatites.

As we observe, new sites were created during the grafting of apatite by tyramine which facilitates the retention of methyl orange. This study first confirmed the effectiveness of grafted apatite by tyramine to retain organic substances, especially colored species. The promising results of the adsorption of MO molecules by the hybrid hydroxyapatite Tyr-HAp could be a good alternative in environment remediation and medical applications.

4. Conclusion

Novel amino-apatites were synthesized using hydrothermal reaction in the presence of different Tyramine contents. The addition of small tyramine amount increases the porosity of grafted apatite by influencing the nucleation and crystalline growth processes of the grafted apatites. Their surface properties were tested by the adsorption of methyl orange and the kinetics are described by pseudo-second-order model. Therefore, there is a good affinity between methyl orange and the grafted hydroxyapatite biomaterials. Further investigations are in progress to
evaluate the hybrid apatite in order to design more efficient hydroxyapatite-based biomaterials and sorbents.

Declaration of Competing Interest

All the authors are aware of the submission and agree to its publication. We confirm that the present submission is original and not under consideration for publication elsewhere. There is no conflict of interest with other people or organization.

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