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Synthesis and characterization of folate-medronate[64Cu] as a new theranostic agent for bone cancer and skeletal diseases

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1. Introduction

A radiopharmaceutical can be understood as any radiolabeled molecule designed for biomedical applications as diagnostic or therapeutic agents. Therefore, radiopharmacy techniques deal with chemical compounds and radioisotopes manipulations, aiming the formulation of a system that is capable of depositing radiation doses only on specific organic tissues [1]. Recent studies indicate that folate has a fundamental role in bone metabolism and in other several types of reactions [2]. This molecule can be incorporated by cells through some carriers that are normally overexpressed on tumor cell membranes [3], which makes it possible to explore folate both to allow tumors to be localized, through diagnostic imaging techniques, and for the specific delivery of therapeutic agents to malignant tissues. Likewise, bisphosphonates are considered one of the main drivers for drug delivery to skeletal tissue. Due to their preferential accumulation in areas with high bone metabolism associated with the mineralization process the bisphosphonates [4], such as the medronate molecule, are used in bone scanning for scintigraphy exams and provide an effective way of diagnosing bone diseases such as primary and metastatic cancer. In this work, the folate-medronate compound synthesized was radiolabeled with copper-64 and investigated as a new theranostic agent for bone cancer and other skeletal diseases. The samples were characterized by FTIR, UV-Vis and XPS. The DFT method was applied for theoretical investigations and the radiolabeling yield was calculated. The results indicate that that folate moiety can be stably bond to medronate molecule and the folate-medronate compound can complex the copper-64 radioisotope, crediting this material for biological investigations.

2. Methodology

The folate-medronate compound was synthesized following our previous work [5]. Briefly, folic acid (1mmol) was dissolved in DMSO followed by adding EDC·HCl (1.2 mmol), NHS (2 mmol) and ethylenediamine (10 mmol). After overnight period, the aminated folate (FA-NH_2) was precipitated by adding acetonitrile and washed by vacuum filtration with acetone (Figure 1a). MDP (1 mmol) was dissolved in DMSO followed by adding EDC.HCl (1.2 mmol), NHS (2 mmol) and FA-NH_2 (1 mmol). After overnight period, the folate-medronate sample (FA-MDP) was precipitated by adding acetonitrile and washed by vacuum filtration with acetone (Figure 2b).

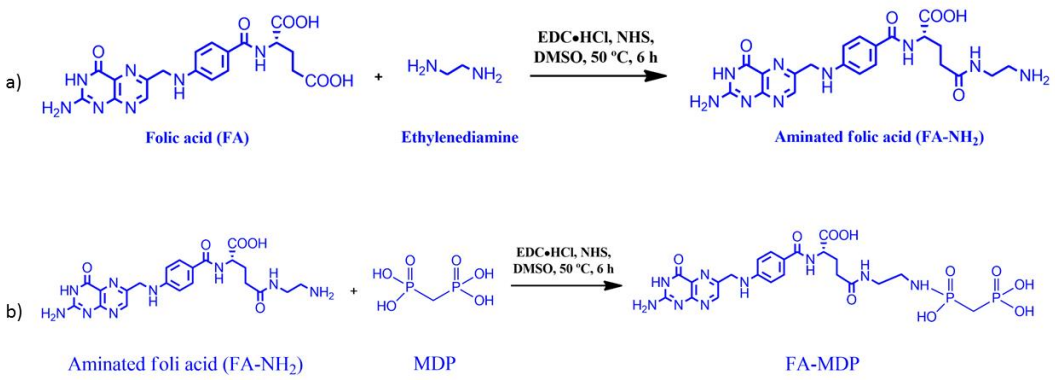

Figure 1: Scheme of the chemical reaction for the folate amination (a) and scheme for the chemical reaction for binding the medronate moiety to the aminated folate molecule (b).

The radiolabeling of FA-MDP compound was achieved by the complexation of copper-64 radioisotope on the phosphate groups from MDP moiety. The complexation was carried out by dissolving the FA-MDP sample in Milli-Q water (1.1 mg/mL) under sonification and subsequently adding sodium bicarbonate (440 mg) to promote the deprotonation of the phosphate groups. Then, 64CuCl3 (1 mCi) was added to a copper (II) chloride solution (1.9 mg/mL) and added to the FA-MDP solution under moderate stirring. After precipitation, the suspension was centrifuged to separate the free ^{64}Cu ions and calculate the radiolabeling yield (63%). The resulting green powder (FA-MDP[^{64}Cu]) was washed and recovered.

The samples were characterized by FTIR, UV-Vis, XPS and the DFT method was applied for theoretical investigations.

3. Results and Discussion

The density functional calculations were performed to obtain insights on the geometry of the FA-MDP[^{64}Cu] sample and on the energetics of the reactions that may lead to a complex system. The deprotonated FA-MDP can then react with Cu^{2+} in an aqueous solution to form a dimeric complex with Cu as evidenced by investigating several possible configurations for FA-MDP[Cu] complexes. To calculate the energy variations, the isomers found with lowest energy was used. The reaction between two deprotonated FA-MDP molecules and one Cu^{2+} ion, a reaction that leads to the dimer FA-MDP-Cu-MDP-FA formation, presents a negative value of ΔU -2.29 eV, showing that the dimer is much more stable than the reactants. Figure 2 shows the most stable configuration found for the FA-MDP-Cu-MDP-FA dimer.

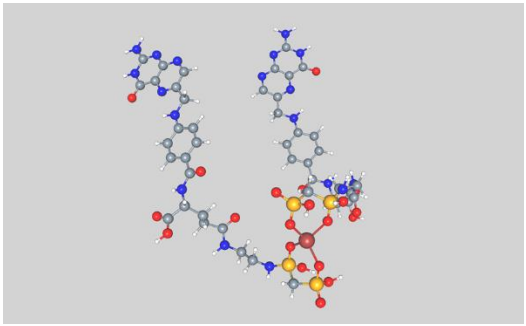

Figure 2: Geometry of the FA-MDP-Cu-MDP-FA complex obtained through DFT calculations. White, red, gray, blue and brown circles represent respectively H, O, C, N, Cu atoms.

The UV-Vis spectroscopy results (Figure 3a) evidenced the charge transfers among the 2p oxygen orbital and the 4s orbital of Cu^{2+} ions around 254 – 264 nm [6] in the FA-MDP[^{64}Cu] spectrum, suggesting the formation of the dimer molecule FA-MDP-Cu-MDP-FA with the complexation of copper ions occurring through the O-Cu-O bond in the R-HOO=P-O-Cu-O-P=OOH-R group as supported by the DFT calculations.

The new band observed at 562 cm^{-1}, found only in the FA-MDP[^{64}Cu] FTIR spectrum (Figure 3b), can be attributed to the Cu-O stretching [7] suggesting that the copper ion complexation in the FA-MDP compound possibly occurs through the formation of the dimer molecule FA-MDP-Cu-MDP-FA, in agreement with the UV-Vis spectroscopy and the DFT calculations. Furthermore, there is a new band only observed in the FA-MDP[^{64}Cu] spectrum at 1260 cm^{-1} that can be attributed to the phosphoryl groups [8], suggesting that the MDP moieties were deprotonated in the FA-MDP[^{64}Cu] sample, as expected.

The XPS survey scan of the FA-MDP[^{64}Cu] sample (Figure 3c) indicates a higher concentration of O and Cu on the surface, indicating a preferred orientation of the complexed molecule. The presence of the strong satellite peaks at the high resolution spectrum of Cu 2p, shown in Figure 3d, clearly indicates that Cu is present in Cu (II) oxidation state. The binding energies of the peaks, centered at 935.5 eV and 955.1 eV, correspond to Cu2p3/2 and Cu2p3/2, while the peaks at 942.0, 944.5 eV and 962.9eV are strong satellites peaks, both of which are a fingerprint of O-Cu-O bonds, suggesting that the complexation of copper ions is occurring through the O-Cu-O bond possibly in the phosphate groups at the extremity of the MDP moiety, in agreement with previous results.

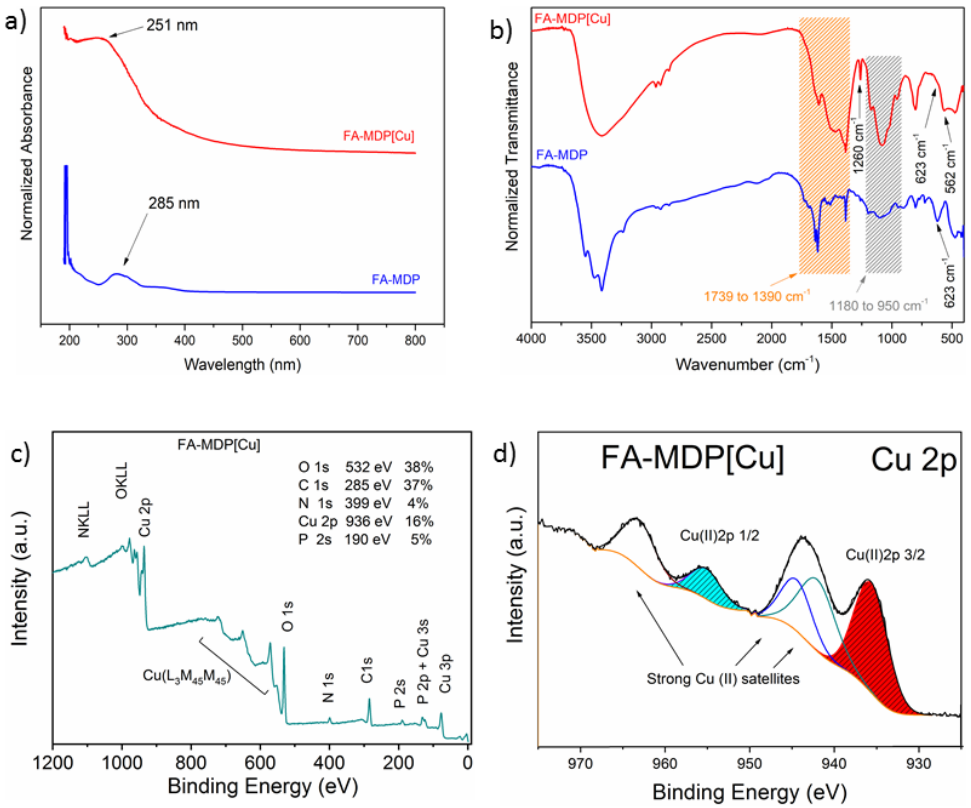

Figure 3: a) UV-Vis spectra of samples FA-MDP and FA-MPD[^{64}Cu]; b) FTIR spectra of samples FA-MDP and FA-MPD[^{64}Cu]; c) XPS survey spectrum of sample FA-MDP[^{64}Cu]; d) XPS high resolution spectrum of Cu 2p photoelectron from sample FA-MDP[^{64}Cu].

4. Conclusions

The results suggest that the radiolabeling process of folate-medronate compound with copper-64 radioisotope leads to a high yield of a stable dimeric radiopharmaceutical, crediting this material for further biological investigations.

Acknowledgements

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