Morin loaded mesoporous molecular sieves as novel devices to the potential treatment of tumor pathologies

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Abstract: Morin is an antioxidant, anti-inflammatory, and anticancer flavonoid extracted from fruits and herbs that may exert beneficial effects for several human pathological conditions. Despite this, the administration of morin represents a challenge due to its low aqueous solubility and high sensitivity. Mesoporous silica materials have emerged as new biocompatible tools for drug delivery, as their pore size can be modulated for maximum surface area to volume ratio. In this contribution, we evaluate the ability of iron-modified mesoporous materials for morin loading and controlled delivery. Methods: The SBA-15 and MCM-41 sieves were synthesized and modified with iron. Characterization by transmission electron microscopy, XRD and UV-Vis revealed adequate pore size and agglomerates of very small metallic nanospecies (nanoclusters), without larger iron oxide nanoparticles. FT-IR spectra confirmed the presence of silanol groups in the solid hosts, which can interact with different groups present in the morin molecules. The incorporation of morin was also corroborated by UV-Vis spectroscopy. Results: SBA-15 materials were more efficient in terms of morin loading capacity due to their larger pore diameter. Finally, biosafety studies using normal epithelial cells revealed that neither the loaded nor the unloaded materials exerted toxicity, even at doses of 1 mg/ml. Conclusions: these findings expand knowledge about mesoporous materials as suitable carriers of flavonoids with the aim of improving therapies for a wide range of pathologies.

Keywords: MESOPOROUS, MORIN, DRUG DELIVERY, ANTIOXIDANT.

1. Introduction

Natural products represent an important source of bioactive compounds. Among them, flavonoids (polyphenols which are found in many fruits and vegetables) have shown efficiency in the treatment of several conditions such as cancer, cardiovascular or neurodegenerative diseases [1]. Morin (3,5,7,2',4'-pentahydroxyflavone), is a flavonol that has been extensively studied for its multiple positive effects on different human diseases. Morin presents, antioxidant, antiinflammatory, antitumoral and antibacterial activities, among others [2]. However, its poor water solubility limits its application in medical treatments [3]. To overcome this difficulty, morin (and other flavonoids) have been loaded into drug carriers [4–6]. By using these systems, the solubility of drugs is enhanced, protecting them from degradation until reaching the region of interest [7]. Different kinds of materials have been studied as drug delivery systems. Among them, mesoporous silica materials (MSM) with highly porous ordered structure are highlighted. MSMs are considered safe (silica is endogenous in human body) and their pore sizes can be modulated between 2 and 10 nm, leading to large specific surface (in the order of 1000 m²/g) and high pore volume (up to 1.3 cm³/g). Such properties are responsible for their high adsorption capacity even for bulky molecules such as many drug molecules. Besides, the surface is versatile for modification with different compounds, like organics compounds and metal ions [8]. The modification with magnetic moieties is very attractive because they offer the alternative of using an external magnetic field to guide material until the region of interest.
In the present work, four mesoporous materials (SBA-15, MCM41, Fe-SBA-15 and Fe-MCM-41) were used to load morin, evaluating the loading efficiency and capability. Besides the release capacity was studied using a media simulating the physiological one. Iron was incorporated in order to obtain a magnetic material. Moreover, morin is a well-known antioxidant compound. Oxidative stress contributes to the pathogenesis of several diseases, including cancer [9]. Antioxidants are known to be capable to fight against oxidative stress. Nonetheless, some limitations exist regarding their use, since after oral administration, their absorption is usually insufficient to get an appropriate concentration in the target site [10]. For that reason, the antioxidant activity of all loaded and unloaded materials was evaluated through the DPPH assay. DPPH, (2,2-diphenyl-1-picrylhydrazyl) is a stable radical with a deep violet color. After treatment with a substance which can donate a proton, this radical transforms into this reduced form, losing its color [11]. Due to its simplicity, this test is one of the most used to test antioxidant activity [12]. Finally, biosafety studies were performed on normal epithelial cells. The set of data achieved within this work is a novel option for the design of drug delivery systems which may ensure multiple function against tumor and other diseases.

2. Materials and Methods

All reagent and solvents were of analytical grade and were used without further purifications. Ferric chloride hexahydrate was provided by Biopack (Argentina). Morin hydrate, DPPH (2,2-Diphenyl-1-picrylhydrazyl), Pluronic P123 (EO20PO70EO20), Tetraethoxysilane (TEOS, 98%), Hydrochloric acid (HCl, 37%) and Sodium hydroxide (NaOH, 97%) were purchased from Sigma Aldrich (USA). Fe(NO₃)₃.9H₂O and Cetyltrimethylammonium bromide (CTABr, 99%) were purchased from Merck.

2.1 Synthesis of mesoporous materials

Metal-free mesoporous molecular sieve SBA-15 was synthesized as previously reported by Zhao et al. [13]. In a typical synthesis, 4 g of Pluronic P123 (Sigma 99%) used as the structure directing agent, were dissolved in 30 g of water and 120 g of 2 M HCl solution under stirring at 35 ºC. Then, 8.5 g of tetraethoxysilane (TEOS) (Aldrich 98%), the silicon source, was added to that solution and kept under stirring at 35 ºC for 20 h. The mixture was aged at 80 ºC overnight without stirring. The solid product was recovered, washed and dried at 60 ºC. In order to remove the organic template, the solid was submitted to a calcination process at 500 ºC for 8 h.

SBA-15 mesoporous molecular sieves modified with iron were synthesized by the direct incorporation of the metal source in the synthesis gel. Pluronic P123 was dissolved in a 2 M HCl solution under stirring at 40 ºC. Then, the iron source, Fe(NO₃)₃.9H₂O, was added to the solution in order to follow a Si/Fe molar ratio of 20 and the stirring was maintained for 30 min. After this period, TEOS was added dropwise to this solution and kept under stirring at 40 ºC for 4 h. Then, the mixture pH was increased to 3.5 adding dropwise ammonia solution (NH₄OH). After the pH stabilization the stirring was maintained 30 min and then the gel was aged without stirring at 40 ºC for 20 h and at 80 ºC for 48 h. After this treatment the solid product was recovered, washed and dried at 60 ºC. Finally, in order to remove the organic template, the solid was submitted to calcination process heating at 1 ºC/min until 500 ºC maintaining this temperature for 8 h. This material was named Fe-SBA-15.

The bare MCM-41 support was synthesized following the method proposed by Gallis et al. [14,15]. Cetyltrimethylammonium bromide (CTAB) as structure directing agent was dissolved in 2 M sodium hydroxide aqueous solution (NaOH) (97%) under heating at 40 ºC and then 10 ml of TEOS was added. The ratios of the reactants...
were as follows: NaOH/Si = 0.50, CTAB/Si = 0.12, water/Si = 132. The resulting mixture (pH 11.25) was stirred at room temperature for 4 h. Then, this gel was heated at 70 °C under stirring. The final solid was then filtered, washed with distilled water and dried at 60 °C overnight. To remove the template, the samples were heated (heating rate of 2 C/min) under N₂ flow up to 500 °C maintaining this temperature for 6 h and subsequently calcined at 500 °C under air flow for 6 h.

The iron-containing MCM-41 type mesoporous materials were prepared by a direct hydrothermal method using CTAB as template, TEOS as silicon source and ferric nitrate (Fe(NO₃)₃·9H₂O) as metal precursor. The pH of the reaction mixture was adjusted to 12 by adding a 2M sodium hydroxide (NaOH) aqueous solution. The catalysts were synthesized from a gel of molar composition: Si/Fe = 20; OH/Si = 0.5; CTABr/Si = 0.12; H₂O/Si = 132. In a typical synthesis, CTAB were dissolved in a NaOH solution in water under agitation at 40 °C. Then, TEOS and the metal source were added to this solution under stirring at 25 °C for 4 h, then maintained 3 h at 70 °C and treated hydrothermally at 100 °C for 1 day under autogeneous pressure using a Teflon-lined stainless-steel autoclave. Finally, this gel was filtered, washed with distilled water and dried at 60 °C overnight. The template was removed from the samples by heating (2 °C/min) under N₂ flow (45 mL/min) at 500 °C for 6 h and then calcinated at 500 °C for 6 h under dry air flow (45 mL/min). This sample was designated as Fe-MCM-41.

2.2 Characterization of materials

X-ray diffraction (XRD) patterns were recorded on a Philips PW3830 diffractometer employing Cu-Kα radiation (λ = 1.5418 Å) in the 2θ range from 20° to 80°. Specific surface area, pore size distribution, and total pore volume were determined from N₂ adsorption–desorption isotherms obtained at 77 K using a Micromeritics ASAP 2010 apparatus. The surface area was determined by the Brunauer–Emmett–Teller (BET) method in the pressure range P/P₀: 0.01–0.21, and pore sizes were determined by The Barrett–Joyner–Halenda (BJH) method. UV/Vis diffuse-reflectance (UV/Vis DR) spectra in absorbance mode were recorded on a JASCO V 650 spectrometer with an integrating sphere, in the wavelength range 200–900 nm.

Transmission electron microscopy (TEM) images were acquired with a TECNAI F20 microscope. Samples were prepared by dispersing a small amount of powder in ethanol and depositing a drop of this emulsion on a formvar-carbon-coated copper grid. The infrared analysis of the samples was recorded on a JASCO 5300 FT-IR spectrometer.

2.3 Morin loading

In a glass, 20 mg of mesoporous molecular sieve and 40 ml of bidistilled water were placed. The suspension was sonicated for 15 minutes. An amount of morin (6 or 9 mg) was dissolved in 5 ml of ethanol and then it was added to the suspension of mesoporous samples. The resulting suspension was magnetically stirred for 24 h. At certain times, aliquots of the suspension were withdrawn and centrifuged to measure the absorption band of morin in UV-Vis spectrophotometer. The concentration of morin in solution was quantified by measuring the absorption band of morin in UV-vis at 364 nm.

2.4 Characterization of morin loaded mesoporous materials.

The incorporation of morin into mesoporous materials was evaluated by Fourier Transform Spectroscopy (FTIR) and UV-vis spectroscopy. For FTIR, samples were mixed with dry KBr powder, compacted and analyzed in a Thermo Scientific Nicolet iS50 in the frequency
range of 400-4000 cm\(^{-1}\). For UV-Vis characterization, samples were diluted in water or in a solution of water: ethanol and measured in a spectrophotometer Shimadzu 160 Japan.

2.5 Antioxidant activity

The antioxidant activity was determined as a function of the discoloration of an ethanolic solution of DPPH 2.8%. Measurements were performed in UV-Vis at 517 nm. Aliquots of 3 ml of DPPH solution were treated with 1 mg of the mesoporous material with and without morin loaded. Then, the suspensions were magnetically stirred for a few seconds and let stand in the dark for 30 minutes. The suspensions were centrifuged to decant the mesoporous molecular sieves and the liquid was measured in UV-Vis spectrophotometer at 517 nm. The percentage of scavenging activity was calculated as follow:

\[
\% \text{ DPPH scavenging} = \frac{Abs_i - Abs_f}{Abs_i} \times 100
\]

Where \(Abs_i\) is the absorbance of DPPH solution at 517 nm before the addition of the material and \(Abs_f\) is the absorbance after the treatment with the mesoporous molecular sieves.

2.6 Cell culture

Vero epithelial cells, derived from African green monkey kidney were acquired from the American Type Culture Collection, Manassas, Virginia (ATCC). The cells were cultured at 37 °C in DMEM (Sigma-Aldrich Chemical Co., St. Louis, Missouri, USA) containing 10% FBS (Internegocios, Argentina), 1% non-essential acids, 100 UI/mL penicillin, 100 mg/mL streptomycin (Gibco, ThermoFisher Scientific, Waltham, MA USA) and 50 mg/mL gentamycin in a humid atmosphere of 5% air CO\(_2\). Cultures were passaged every 3 days with fresh medium. All experiments were performed using passages less than 10. Experimental cultures were grown to 80% confluence in serum-containing medium.

2.7 Cytotoxicity assay

The analysis of the toxicity of the mesoporous materials was made through the Neutral Red Uptake test (NRU), as indicated in ISO-10993-5-2009 International Standard. Briefly, 1x10\(^5\) Vero cells were seeded six times for each condition in sterile 96-well plates and allowed to grow for 24 hours. Then, the cells were treated for 24 hours with increasing concentrations of mesoporous materials in DMEM 10% SFB. As control conditions, the cells were incubated with the culture medium and 1% hydrogen peroxide was the positive control of cell death. After treatment, the cells were washed with phosphate buffer pH 7.4 at 37°C and incubated with Neutral Red medium (NR medium) (1 ml stock solution of 3% Neutral Red, from Santa Cruz Biotechnology, per 79 ml of DMEM 3% SFB). The concentration of fetal serum was reduced to 3% to avoid reading absorbance interferences. After 3 hours of incubation with the NR medium, the cells were washed with phosphate buffer and the dye incorporated by the cells was extracted with extraction medium (1% acetic acid, 50% distilled water, 49% ethanol 96°). The absorbance at 540 nm was measured in a Biotek Synergy HT multi-plate reader.

3. Results and Discussion
3.1. Mesoporous molecular sieves characterization

**Figure 1** depicts the low angle XRD patterns for the synthesized solids. MCM-41 and Fe-MCM-41 samples exhibit in their XRD patterns (**Figure 1A**) a peak of high intensity at 2θ ~ 2.5° and two lower intensity peaks at 2θ <10 °, corresponding to a one-dimensional hexagonal arrangement of channels typical of MCM-41 structure. For its part, the SBA-15 and Fe-SBA-15 samples display well-resolved XRD lines (**Figure 1B**) showing a peak of high intensity at 2θ < 1° and two lower intensity peaks at 2θ <3°, corresponding to the diffraction planes usually assigned to a hexagonal pore arrangement of SBA-15 structures.

![Figure 1](image-url)

**Figure 1.** Low angle XRD patterns of the synthesized samples: A) samples of MCM-41 type and B) samples of SBA-15 type.

The XRD patterns at high angle for all samples have not been presented since they did not show peaks, evidencing that if the oxide nanospecies are present they are amorphous or their crystal domain size is below the XRD detection limit.

The N₂ adsorption-desorption isotherms of MCM-41 and Fe-MCM-41 samples are shown in **Figure 2A**. The materials exhibit type IV isotherms with an inflection at relative pressures of p/p₀~0.2-0.3 characteristic of capillary condensation inside the conventional mesopores present in MCM-41 structure, called primary mesopores [16,17]. The presence of hysteresis loops in type IV isotherms is related with the shape and size of the pores in the adsorbent [18]. In this sense, the negligible hysteresis observed for isotherms of the Fe-MCM-41 sample shows that the method of incorporation of the metal used allowed the formation of uniform pores, similar to those of pure silica matrix. Moreover, the Fe-MCM-41 sample exhibited an increase in the adsorption branch at P/P₀~0.80-0.85 which is usually associated to capillary condensation in secondary mesopores, generally interstitial pores.

**Figure 2B** shows the N₂ adsorption-desorption isotherms of the SBA-15 and Fe-SBA-15 samples; both materials exhibit isotherms of type IV with hysteresis H1. According to bibliography, this hysteresis occurs for a N₂ adsorption in cylindrical pores when the pore width exceeds a size of ~ 4 nm. The final saturation plateau observed for both samples would be give account for the well pore alignment characteristic of highly ordered molecular sieves, even when the iron is present in the synthesis gel [19].
Meanwhile, Figure 3A and 3B show that all the samples have a narrow pore diameter distribution corresponding to the primary mesoporous range.

Table 1 reports the physicochemical properties of the synthesized solids. The free metal and Fe modified SBA-15 and MCM-41 samples exhibit high specific surfaces typical of these highly ordered mesoporous materials. Although all the samples have high surface areas, the main features that distinguish the MCM-41 materials of SBA-15 are the pore diameter and volume, which are significantly greater for the latter case. This would give to SBA-15 type materials greater capacity to accommodate organic molecules inside their pores.
Table 1: Surface area, pore diameter, pore volume, and metal content of the samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Area [m²/g]</th>
<th>Pore Diameter [nm]</th>
<th>Pore Volume [cm³ g⁻¹]</th>
<th>Metal Content [wt. %]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCM-41</td>
<td>996</td>
<td>3.5</td>
<td>0.70</td>
<td>-</td>
</tr>
<tr>
<td>Fe-MCM-41</td>
<td>765</td>
<td>3.7</td>
<td>0.68</td>
<td>6.27</td>
</tr>
<tr>
<td>SBA-15</td>
<td>893</td>
<td>7.0</td>
<td>1.10</td>
<td>-</td>
</tr>
<tr>
<td>Fe-SBA-15</td>
<td>897</td>
<td>6.0</td>
<td>0.80</td>
<td>4.02</td>
</tr>
</tbody>
</table>

* Determined by BET. b Pore diameter determined by the NLDFT method. c Determined by o-phenantroline technique.

**Figure 4** shows UV-Vis DR spectra of both sample types synthesized with iron. The strong absorption around 260 nm indicates the incorporation of metal into the framework. As it was claimed by us in a previous report [16], the iron in the samples modified by the direct incorporation method, is mostly incorporated into the framework in distorted tetrahedral coordination. During the calcination process, the migration of these iron ions may occur from within the framework to the wall of the pores or onto the external surface, forming oxide nanoparticles or nanoclusters. Although they were not observed by XRD at high angle, the UV-Vis absorption extending to longer wavelengths would be giving evidence that the iron is also present in octahedral coordination in extra-framework positions as nanoclusters or nanoparticles [20–23].

Although the Fe-MCM-41 and Fe-SBA-15 samples showed similar spectra (see **Figure 4A** and **B**) the major presence of nanoclusters for the latter sample is evidenced by the higher absorption at around 300-400 nm. This feature could be favored by the synthesis conditions employed for SBA-15 structures.

![Figure 4: UV-Vis DR spectra of the synthesized samples A) Fe-MCM-41 B) Fe-SBA-15.](image)

Transmission electron microscopy (TEM) was used to extend the physicochemical analysis presented by XRD and UV-Vis DR. **Figures 5A** and **5B** mainly correspond to views perpendicular to the direction of the pore arrangement where unidirectional straight channels arranged along the long axis can be observed. In addition, both samples show irregular contrasts in the images, which are possible to consider as agglomerates of very small metal nanospecies (nanoclusters); nevertheless, no iron oxide nanoparticles of larger size were observed on the surface. This analysis is according to the inferred by XRD and UV-Vis DR. In the inset of **Figure 5B** a frontal view of the arrangement of
mesopores can be seen for the Fe-SBA-15 sample after Morin adsorption. Here it is very noticeable that the regular order of the channels was preserved after the drug adsorption.

![Figure 5: A) Fe-MCM-41 B) Fe-SBA-15 (Inset: Fe-SBA-15 after Morin adsorption)](image)

The FT-IR spectra of the synthesized samples were recorded after vacuum application at 400 °C (Figure 6) for eliminate the adsorbed environment water. It is known that the band at 3740 cm\(^{-1}\) indicates the presence of silanols groups on the solid host which can interact with different groups present in the drug molecules. Therefore, it is very important to know the availability of silanols groups in all samples [24,25]. Here, it is noteworthy that this band is very intense for both pure matrices and matrices modified with iron by the direct method employed by us in this study. Meanwhile other methods, such as wet impregnation, tend to decrease the density of silanols due to Si–OH groups can be blocked after the condensation with Fe species, leading to the formation of the Si–O–Fe bonds onto the walls and consequently to the lack of the band corresponding to silanol groups [26]. In addition although the Fe-SBA-15 solid shows a higher proportion of nanoclusters, this was not sufficient to provoke the blocking of silanol groups. Therefore, judging for the similar presence of silanol groups in all samples synthesized in this work, it is expectable that they can present a good adsorption capacity.
Figure 6: FTIR spectra of the synthesized samples

3.2 Drug adsorption studies

The evaluation of the capacity of the mesoporous molecular sieves to act as a carrier of morin, was performed using two different amounts of drug (6 or 9 mg). The experiments were carried out with the four materials. The obtained results, expressed as loading efficiency (LE%) and loading capability (LC%) are depicted in Table 2.

Table 2. Loading efficiency and capacity of different formulations employing initially 6 and 9 mg of morin.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of morin used: 6 mg</th>
<th>Amount of morin used: 9 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LE%</td>
<td>LC%</td>
</tr>
<tr>
<td>MCM-41</td>
<td>74</td>
<td>21.7</td>
</tr>
<tr>
<td>Fe-MCM-41</td>
<td>71</td>
<td>21.5</td>
</tr>
<tr>
<td>SBA-15</td>
<td>80</td>
<td>23.4</td>
</tr>
<tr>
<td>Fe-SBA-15</td>
<td>80</td>
<td>24</td>
</tr>
</tbody>
</table>

It can be seen that SBA-15 and Fe-SBA-15 materials, whose pores are greater in diameter and volume than the pores of MCM-41 and Fe-MCM-41, load a major percentage of morin. The loading efficiency was not affected by the amount of morin used in the experiment, while the loading capability increases with the amount of morin. That means that more mg of the drug was adsorbed as the amount of the drug used increased.

The incorporation of morin into mesoporous materials was confirmed by IR spectrometry, as it is shown in Figure 7. The presence of the flavonol is particularly evident in the region between 1521 and 1177 cm\(^{-1}\) where the bands associated with C-C, O-C stretching and with the in-plane bending C-C-H, C-O-H, C-C-O and C-C-C of the rings are distinguished [27]. The presence of the drug was also confirmed through UV-Vis spectroscopy. The molecule of morin,
presents two absorption bands, one at 264 nm corresponding to the benzoyl function (ring B, see inset Figure 7B) and the other at 395 nm corresponding to the cinnamoyl moiety (rings A and C)[28]. Figure 7B compares the UV-vis spectra of morin, MCM-41/morin and Fe-MCM-41/morin. In both materials, the adsorption band of the flavonol is evident, although with a slight bathochromic shift. Interactions of the drug with iron could be responsible of this shifting [29].

![Figure 7. A) Comparison of FTIR spectrum of Fe-MCM-41, morin and Fe-MCM-morin. B) Comparison of UV-Vis spectra of morin and Fe-MCM-41-morin.](image)

Morin could interacted with the silanol groups through hydrogen bonding, because of the multiple OH groups present in the structure of the flavonoid [30,31]. In the case of iron-modified materials, an interaction between morin and the metal is also possible. It has been reported that morin forms complex with iron through the 3-hydroxyl-carbonyl and 5-hydroxyl-carbonyl groups in the pH range from 3 to 7 [32]. Since the pH range of adsorptions was 5 to 6 the formation of the complex could took place.

Release experiments were carried out at pH 5 and 37°C and followed for 48 hours.

A burst release effect was observed after the first hour. The process hardly varies after this time, as it is shows in Table 3. The release percentage is small for all materials, which may be due to a strong interaction between the flavonol and the material [33].

The materials with the lowest pore diameter present lower drug release at the beginning, probably, because drug diffusion was hampered through the narrow pores. With time, MCM-41 reaches a release percentage similar to SBA-15 and Fe-SBA-15. Previous work with indomethacin has shown that it releases is faster and in mayor percentage from Fe-MCM-41 than MCM-41 because the presence of nanocluster hinders a deep penetration of the molecule into the channels [26]. However, when working with morin, Fe-MCM-41 present the lowest release. This observation could be related with iron the formation of a complex morin-iron. Table 1 shows that the percentage of the metal is higher for Fe-MCM-41 than for Fe-SBA-15, that means that a major percentage of molecules could be complexed. As a result, the release of the flavonoid from Fe-MCM-41 is the lowest.
Table 3. Release of Morin versus time for each formulation.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>MCM-41</th>
<th>Fe-MCM-41</th>
<th>SBA-15</th>
<th>Fe-SBA-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15%</td>
<td>10%</td>
<td>18.00%</td>
<td>19%</td>
</tr>
<tr>
<td>2</td>
<td>15%</td>
<td>6%</td>
<td>18%</td>
<td>20%</td>
</tr>
<tr>
<td>4</td>
<td>18%</td>
<td>9%</td>
<td>19%</td>
<td>25%</td>
</tr>
<tr>
<td>24</td>
<td>21%</td>
<td>6%</td>
<td>20%</td>
<td>28%</td>
</tr>
<tr>
<td>48</td>
<td>30%</td>
<td>8%</td>
<td>20%</td>
<td>37%</td>
</tr>
</tbody>
</table>

3.3 Antioxidant activity
The antioxidant activity of the samples was evaluated using the DPPH assay. The ethanolic solution of this free radical is deep violet and has an absorption band at 517 nm. In contact with an antioxidant substance, the solution starts to be discolored and the band at 517 nm decreases. The scavenging activity is then quantified as it was explained in Methods section.

As it can be seen in Figure 8, the mesoporous MCM-41 and SBA-15 did not show antioxidant activity, in agreement with previous reports [34,35]. However, after the adsorption of morin the activity significantly increased. On the other hand, Fe-MCM-41 and Fe-SBA-15 present small scavenging activity and it did not improve after the incorporation of the flavonoid. This finding supports the idea of the complexation of morin with iron. Markovic et al reported that iron-morin complex is not an effective reductor of DPPH [32], presumable because the same structures involved in iron complexation are important for the antioxidant activity.

As it was mentioned, Fe-SBA-15 and Fe-MCM-41 showed better scavenger activity compared with SBA-15 and MCM-41. This difference could be due to the presence of iron oxides. In fact, the value achieved for the iron-modified materials is similar to the result that we have reported for raw magnetite [36].

Figure 8. Antioxidant activity of materials before and after morin loaded.

3.4 Biocompatibility analysis
An important step in validating materials to improve drug delivery is to analyze whether these carriers can exert cytotoxic effects on normal cells. To evaluate this parameter, we chose Vero cells, a cell line widely used in biomedicine for the in vitro screening of cytotoxic effects induced by new designed compounds [37]. Besides, we selected NRU assay, since this test is one of the most reliable for the detection of variations on cell viability. This assay is based on the ability of viable cells to incorporate the supravital Neutral Red dye into lysosomes. The amount of retained dye is proportional to the number of viable cells. Therefore, it is possible to distinguish between viable, damaged, or dead cells according to their specific lysosomal ability to absorb the dye [38]. In order to detect possible deleterious effects associated with our materials, we treated Vero cells with Fe-MCM-41, Fe-MCM-41/morin, SBA-15 and SBA-15/morin for 24 h in a range of concentrations of 50 μg/ml to 200 μg/ml. The doses were according to recent research on similar mesoporous materials [39]. As shown in Figure 9, no changes were detected in the absorbance measured after the treatments at all doses of materials after 24 hours concerning the cells incubated with the culture medium alone. Instead, a highly significant reduction in optical density was seen when the cells were exposed to hydrogen peroxide. These data indicate that the materials Fe-MCM-41 and SBA-15 with or without loaded morin do not exert toxicity on this normal epithelial cell line derived from the kidney.

![Figure 9. Citotoxicity evaluation of mesoporous materials.](image)

Previous research has shown that SBA-15 mesoporous materials loaded with quercetin, another natural flavonoid like morin, can exert toxicity when are administered to HUT-78 tumor cells, derived from lymphoma. However, in line with our results, alterations in the cell proliferation rate were not detected when non-malignant HEK-293 cells were exposed to quercetin-loaded SBA-15 materials, even at doses of 1 mg/ml [39]. Similar findings were reported more recently with SBA-16 Mg-functionalized mesoporous silicas loaded with morin. In this work, Trendafilova and colleagues evidenced that the morin-loaded formulations did not inhibit the proliferation of normal HEK-293 cells even at high concentrations [35]. Together, these findings expand the knowledge on the biosafety of mesoporous materials, as suitable carriers for flavonoids with the objective of improved anti-cancer therapies with reduced risks and systemic toxicity for oncologic patients.
4. Conclusions

Four mesoporous materials were tested as morin delivery systems. Absorption studies showed that the materials with greater pores (SBA-15 and Fe-SBA-15) exhibited better loading efficiency because they have a better capacity to accommodate molecules inside their structural channels. Yet, the percentage of morin adsorbed by MCM-41 and Fe-MCM-41 is also high. Up to this point, the four materials can be considered as good delivery systems for morin. However, in iron modified materials morin can complex with the metal. This situation is particularly important for Fe-MCM-41, where the major presence of iron could complex a greater number of molecules. This, in addition to a small pore diameter from where the diffusion of the drug is hindering, could explain the very low release percentage of morin.

The notion of a complex formation with iron was supported by the results obtained with DPPH test to evaluate antioxidant property. The iron modified materials did not improve their scavenging activity after morin adsorption. The biosafety of SBA-15 and a metal modified material (Fe-MCM-41), with and without morin were assayed. These materials did not exert any toxicity in normal epithelial cell line derived from kidney.

Although biological activity of these materials against malignant cells was not performed, the results obtained can be taken as preliminary proofs that SBA-15 and MCM-41 could be suitable delivery systems for morin. The presence of iron, as mentioned, affected the antioxidant activity of the flavonol, a function which is very important to treat pathologies such as cancer.

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References


