Aluminium doped MCM-41 nanoparticles as platforms for the dual encapsulation of a CO-releasing molecule and *cisplatin*

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ABSTRACT

Mesoporous silica Al-MCM-41 nanoparticles have been used, for the first time, as vehicles for the single and dual encapsulation of the cationic CO-releasing molecule (CORM) [Mn(1,4,7-triazacyclononane)$(CO)_3]^+$ ($ALF472^+$) and the well-known antineoplastic drug, *cis*-$[PtCl_2(NH_3)_2]$ (*cisplatin*). Thus, two new hybrid materials, namely ALF472@Al-MCM-41 and ALF472-*cisplatin*@Al-MCM-41 have been isolated and fully characterized. The results reveal that the presence of CORM molecules enhances *cisplatin* loading threefold, yielding a cargo of 0.45 mmol g^{-1} of $ALF472^+$ and 0.12 mmol g^{-1} of the platinum complex for ALF472-*cisplatin*@Al-MCM-41. It is worth noting that ALF472@Al-MCM-41 shows a good dispersion in phosphate-buffered saline solution, whilst the dual hybrid material slightly aggregates in this simulated physiological medium (hydrodynamic size: 112 ± 23 and 336 ± 50 nm, respectively). In addition, both hybrid materials (ALF472@Al-MCM-41 and ALF472-*cisplatin*@Al-MCM-41) behave as photoactive CO-releasing materials, delivering 0.25 and 0.11 equivalents of CO, respectively, after 24 hours and exhibiting a more controlled CO delivery than that of the free CORM. Finally, metal leaching studies have confirmed the good retention capacity of Al-MCM-41 towards the potentially toxic manganese fragments (86 % of retention after 72 hours) as well as the low release of *cisplatin* (*ca.* 7 % after 72 hours).

INTRODUCTION

In spite of the well-known toxicity of CO at medium-high concentrations, recent investigations have shown that this gas can be exploited as a therapeutic principle, mainly in inflammatory processes and cardiovascular diseases as well as in transplantation and preservation of organs.[1] In this context, the main drawbacks for its practical medical use derive from its lack of tissue specificity and the need for in-hospital controlled administration of the gas. In order to overcome these issues, different molecules capable of releasing CO (CORMs) in a triggered manner have emerged in the last two decades.[2–6] In particular, special attention has been drawn to those showing a turn on-off mechanism of CO delivery in the presence of light.[4,7,8] However, these photoactive CO-releasing molecules have limited stability in biological media and their resulting photoproducts may exert undesirable toxic effects. To tackle this problem, a wide range of vehicles have been explored as platforms for these photoCORMs, leading to advanced solids with CO releasing properties upon irradiation (photoCORMAs).[9–17]Amongst these carriers, inorganic porous scaffolds, such as mesoporous silicas and metal-organic frameworks (MOFs), have recently emerged as promising candidates for this application.[18–22] The main advantages of the use of these inorganic platforms are the protection of the carbonyl metal complexes towards their premature degradation in physiological media, as well as the trapping of the corresponding toxic decarbonylation fragments within their matrixes. In this context, Mascharak *et al*. published the first example of a photoCORMA based on a mesoporous silica. In particular, they used the doped aluminium MCM-41 (Al-MCM-41) as nanocarrier of the photoactive CORM, $[Mn(pqa)(CO_3)]^+$ (pqa = (2-pyridylmethyl)(2-quinolylmethyl)amine), demonstrating the capacity of this hybrid system to relax rat aorta muscle rings in tissue bath experiments.[20] In a later study, they designed a trackable light triggered CO-delivery material, with cytotoxic activity against

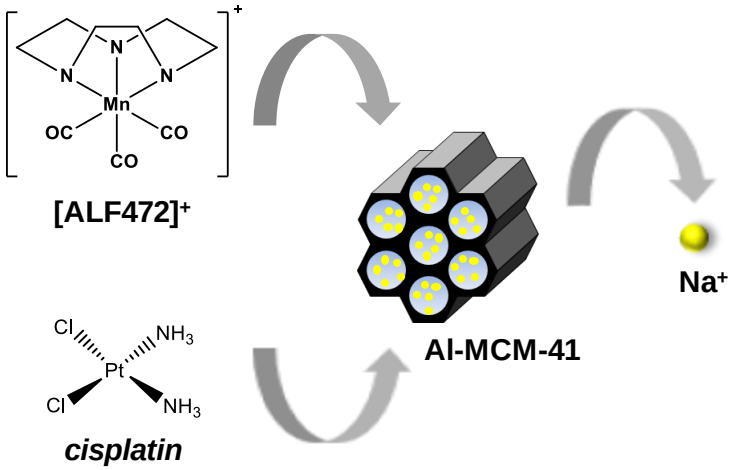
human breast-cancer cells, by combining the same silica matrix (Al-MCM-41) and the rhenium photoCORM, $[Re(CO)_3(pbt)(PPh_3)]^+$ (pbt = 2-(2-pyridyl)-benzothiazole).[21] To the best of our knowledge, only one other paper has been published to date regarding the use of mesoporous silicas as platforms for the preparation of CORMAs.[18]Specifically, our group loaded the cationic photoCORM $[Mn(tacn)(CO)_3]^+$ (tacn = 1,4,7-triazacyclononane) in the functionalized MCM-41-SO_3H and SBA-15-SO_3H silicas. However, the resulting materials showed a low CORM retention in PBS (Phosphate Buffered Saline), probably due to cation exchange processes with this physiological buffer. Therefore, taking into account all these results, Al-MCM-41 can be considered a promising platform for the preparation of CORMAs, as it is a highly biocompatible mesoporous material, which exhibits a remarkable stability in physiological medium, and can be prepared as nanoparticles in reproducible manner.[23] These features, together with the anionic nature of its skeleton, make it a good candidate to encapsulate and strongly retain the high payload of cationic species, such as cationic CORMs, as has been previously demonstrated by the Mascharak research group.[20,21]

In another context, Inorganic Chemistry has made its most important contribution towards fighting cancer by developing platinum complexes, which currently provide highly effective chemotherapy for testicular, ovarian, neck, cervical and oesophageal cancers.[24] One of these platinum complexes, *cis*-diamminedichloroplatinum(II) (*cisplatin*) is the world's most widely used chemotherapeutic drug and has had a profound effect on the treatment of testicular cancer, making it a model for a curable tumour. However, platinum complexes experience loss of activity and side effects due to poor circulation and delivery to the tumour, and high reactivity with other molecules prior to reaching their primary biological target (DNA). In this context, the

use of different biocompatible vehicles for platinum drugs may allow the active principle to reach intact the target cells in need of treatment.[25]

It has also been proven that carbon monoxide can exert a cytoprotective action towards healthy cells,[1] which may be useful for the mitigation of undesirable side effects (namely, cardiotoxicity, nefrotoxicity, etc.) associated to the administration of chemotherapeutic treatments. Thus, Piantadosi *et al*. demonstrated that CO-inhalation ameliorated doxorubicin cardiomyophaty in mice,[26] whilst Kim *et al*. reported the protective effect of CO *in vitro*, as inhibitor of the doxorubicin-induced apoptosis in H9c2 cardiomyocytes.[27] Additionally, Motterlini *et al.*[28] confirmed that the co-administration of *cisplatin* and CORM-3 decreased the *in vitro* cytotoxicity of this antineoplastic agent against LLC-PK_1 renal epithelial cells. Likewise, they demonstrated the benefit of using CORM-3 *in vivo*, as a therapeutic adjuvant in the treatment of *cisplatin*-induced nephrotoxicity.

Taking into account this background, in this study we have taken advantage of the good retention properties of Al-MCM-41 towards cationic species to prepare, for the first time, a new CORMA encapsulating two bioactive molecules with complementary therapeutic properties. With this aim, we have performed the dual loading in Al-MCM-41 of the water soluble and air stable cationic CO-releasing molecule, $[Mn(tacn)(CO)_3]^+$ (tacn = 1,4,7-triazacyclononane), and the well-known anticancer drug *cis*-$[PtCl_2(NH_3)_2]$, *cisplatin* (**Scheme 1**). In addition, both metal leaching and CO-releasing kinetics studies of the resulting hybrid material has been evaluated in simulated physiological conditions.



Scheme 1: Dual encapsulation strategy of the cationic CORM ALF472^{+} ([Mn(tacn)(CO)$_3$]$^{+}$, tacn = 1,4,7-triazacyclononane) and the anticancer drug *cisplatin* (*cis*-[PtCl$_2$(NH$_3$)$_2$]) in the aluminium doped mesoporous silica Al-MCM-41.

EXPERIMENTAL SECTION

All chemicals were commercially available and used without further purification.

Synthesis of Al-MCM-41.

The mesoporous silica Al-MCM-41 was synthesized as previously reported,[20] based on the procedure first described by Cai *et al*.[29] In a typical synthesis, 1 g of cetyltrimethylammonium bromide (CTAB) was dissolved at 80 ºC in 480 mL of water and 3.5 mL of 2 M NaOH solution. Afterwards, 98 mg of $NaAlO_2$ and 4.52 mg of tetraethyl-orthosilicate (TEOS) were added, and the reaction was kept at 80 ºC for 2 hours. The white powder was filtered, washed with water and dried at room temperature. The surfactant was removed by calcination heating at 550 ºC for 5 hours with a ramp rate of 1 ºC min^{-1}. Anal. Calcd. for

$SiO_2(AlO_2Na)_{0.106}(C_{19}H_{42}BrN)_{0.0065}(H_2O)_{0.45}$ (Al-MCM-41): C 1.15; H 1.44; N 0.19; Found: C 1.47; H 1.49; N 0.14. Residue after thermal treatment of Al-MCM-41: SiO_2 $(Al_2O_3\ Na_2O)_{0.053,}$ calculated: 86.8 %; found 88.1 %. Al/Si ratio determined by EDX: 0.106.

Synthesis of cis-[diamminedichloroplatinum(II)], cis-[$PtCl_2(NH_3)_2$] (cisplatin). The compound was synthesized as previously reported.[30] All the manipulations were carried out in absence of light. In a typical reaction, 500 mg (1.2 mmol) of K_2PtCl_4 was dissolved in 5 mL of water. Then, 828 mg (5 mmol) of KI was added and the mixture was stirred for 10 minutes. Afterwards, 0.4 mL of a mixture of H_2O:NH_3 (1:1) was incorporated and the resulting solution was kept under stirring for 30 minutes. As a result, a precipitate of [$PtI_2(NH_3)$] was obtained, which was filtered, washed with water (3 x 5 mL) and collected. Then, a solution of $Ag(NO_3)$ (373 mg (2.2 mmol) in 10 mL of water) was mixed with 530 mg (1.1 mmol) of [$PtI_2(NH_3)$] and kept at 70 ºC for 1.5 hours. The solid was filtered and 196 mg (2.6 mmol) of KCl was added to the solution and kept at 70 ºC for 1 hour. The yellow precipitate (*cisplatin*) was filtered, washed with water (3 x 10 mL), dried with diethylether (3 x 10 mL) and collected. Anal. Calcd. for *cis*-[$PtCl_2(NH_3)_2$] (*cisplatin*): H 2.06; N 9.34; found: H 2.14; N 9.13. Residue after thermal treatment of *cisplatin*: Pt, calculated: 65.02%; found: 65.07%.

Synthesis of [1,4,7-(triazacyclononane)tricarbonylmanganese(I)bromide], [$Mn(NC_2H_4)_3(CO)_3$]Br (ALF472). The metal complex first described by Pomp *et al*.[31] was used as received from Alfama Ltd. Spectroscopic data for the analytically pure compound not given in the original publication: FITR (KBr pellets) ν_{CO} = 2017 and 1895 cm^{-1}; ^1H NMR, 400 MHz, δ ppm) (D_2O) : 2.75 (m, CH_2), 3.07 ppm (m, CH_2), 6.07 ppm (NH). Solubility in water (5 mg mL^-

[1]). Anal. Calcd. for $[Mn(HNC_2H_4)_3(CO)_3]Br$ (ALF472): C 31.06; H 4.34; N 12.07; Found: C 31.41; H 4.04; N 12.65 %.

Evacuation/Activation of Al-MCM-41. Prior to the loading of ALF472, the mesoporous silica material was outgassed (10^{-4} bar, 150 °C) for 24 hours. Under these conditions, the complete removal of the solvent guest molecules was achieved and empty pores ready for drug adsorption were obtained.

Single encapsulation of ALF472 into Al-MCM-41. The incorporation of ALF472 into the mesoporous silica material was carried out by a solid-liquid impregnation method. In a typical experiment, 100 mg of activated Al-MCM-41 (1.45 mmol, equivalent to 0.15 mmol of exchangeable cationic sites) was suspended in a 10 mL aqueous solution of ALF472 (103.3 mg, 0.30 mmol). The mixture was stirred and kept at room temperature and in darkness for 1 day in order to assure that equilibrium was reached. Afterwards, the sample was centrifuged (3500 rpm / 5 min) and the resulting solid was washed with water (3 x 10 mL) and dried at room temperature. Anal. Calcd. for $SiO_2(AlO_2Na)_{0.059}(AlO_2[Mn(HNC_2H_4)_3(CO)_3])_{0.037}(H_2O)_{0.65}$ (ALF472@Al-MCM-41): C 4.51; H 2.11; N 1.75; Found: C 4.14; H 1.82; N 2.00. Residue after thermal treatment of ALF472@Al-MCM-41: $(SiO_2(Al_2O_3)_{0.048})(Na_2O)_{0.030}(MnO)_{0.037})$, calculated: 78.1%; found: 73.9%. Ratio (EDX): Si/Mn: 0.030.

Dual encapsulation of ALF472 and cisplatin into Al-MCM-41. The incorporation of both drugs was carried out in only one step, by dissolving 15 mg (0.05 mmol) of *cisplatin* and 53.5 mg (0.15 mmol) of ALF472 in 15 mL of water. Then 50 mg of activated Al-MCM-41 (0.72 mmol,

equivalent to 0.08 mmol of exchangeable cationic sites) were added to the solution. The mixture was stirred and kept at room temperature and in darkness for 1 day in order to assure that equilibrium was reached. Afterwards, the sample was centrifuged (3500 rpm / 5 min) and the resulting solid was washed with water (3 x 15 mL) and dried at room temperature. Anal. Calcd. for $SiO_2(AlO_2Na)_{0.059}(AlO_2[Mn(HNC_2H_4)_3(CO)_3])_{0.036}[PtCl_2(NH_3)_2]_{0.01}(H_2O)_{1.85}$ (ALF472-*cisplatin*@Al-MCM-41): C 3.44; H 3.83; N 1.58; Found: C 3.60; H 3.34; N 1.53. Residue after thermal treatment of ALF472-*cisplatin*@Al-MCM-41: $(SiO_2(Al_2O_3)_{0.048})(Na_2O)_{0.030}(MnO)_{0.036}Pt_{0.01}$), calculated: 63.1%; found: 58.0%. Ratio (EDX): Mn/Pt: 3.00.

Single encapsulation of cisplatin into Al-MCM-41. The incorporation of *cisplatin* into the mesoporous silica material was also carried out by a solid-liquid impregnation method. In a typical experiment, 50 mg of activated Al-MCM-41 (0.72 mmol, equivalent to 0.08 mmol of exchangeable cationic sites) was suspended in a 15 mL aqueous solution of *cisplatin* (15.0 mg, 0.05 mmol). The mixture was stirred and kept at room temperature and in darkness for 1 day in order to assure that equilibrium was reached. Afterwards, the sample was centrifuged (3500 rpm / 5 min) and the resulting solid was washed with water (3 x 15 mL) and dried at room temperature. Anal. Calcd. for $SiO_2(AlO_2Na)_{0.106}[PtCl_2(NH_3)_2]_{0.003}(C_{19}H_{42}NBr)_{0.003}(H_2O)_{1.1}$ (*cisplatin*@Al-MCM-41): C 0.76; H 2.61; N 0.14; Found: C 0.75; H 2.60; N 0.20. Residue after thermal treatment of *cisplatin*@Al-MCM-41: $(SiO_2(Al_2O_3Na_2O)_{0.053}Pt_{0.003})$, calculated: 76.6%, found: 77.8%.

Hydrodynamic size and Zeta-potential measurements. 5 mg of Al-MCM-41, ALF472@Al-MCM-41, *cisplatin*@Al-MCM-41 or ALF472-*cisplatin*@Al-MCM-41 was suspended in 10 mL

of PBS (10 mM, pH 7.4). The suspensions were homogenized before DLS and *Z*-potential measurements by sonication (15 min, pulse on/off: 15 s, amplitude 30 %, 20 W).

CO delivery studies. The amount of released CO was monitored as previously reported by others.[8,32] Specifically, the CO delivery from free ALF472 and the loaded materials ALF472@Al-MCM-41 and ALF472-*cisplatin*@Al-MCM-41 were studied spectrophotometrically by measuring the conversion of deoxy-myoglobin (deoxy-Mb) to carbonmonoxy-myoglobin (Mb-CO). The amount of formed Mb-CO was quantified by measuring the absorbance at 423 nm at different times. Stock solutions of myoglobin from equine heart (100 µM, adjusted by UV-visible spectroscopy), sodium dithionite (40 mg mL^{-1}), ALF472 (1 mM) and stock suspensions of the CO-releasing materials, with a final concentration of 1 mM based on ALF472 (ALF472@Al-MCM-41: 2.4 mg mL^{-1}, ALF472-*cisplatin*@Al-MCM-41: 3.1 mg mL^{-1}), were freshly prepared in degassed Phosphate Buffered Saline (PBS, 10 mM, pH 7.4). In a typical experiment, 100 µL of myoglobin stock solution, 100 µL sodium dithionite stock solution, 790 µL of degassed PBS and 10 µL of ALF472 stock solution or CO-releasing material stock suspensions were added in a sealed quartz cuvette (1400 µL) under inert atmosphere and kept at 37 °C under visible light (luminous flux: 600 lm; color temperature: 4000 K, distance between lamp and cuvette: 5 cm) for 24 hours. The solutions were analysed by means of UV-vis at different times (0, 15, 30, 60, 90, 120, 150, 180, 240, 300, 360, 480, 600, 720 and 1440 minutes) in order to determine the released amount of CO and the kinetics of the process. At the end of each experiment, the solutions were bubbled with CO and the concentration of Mb-CO was calculated as previously reported.[32] All the experiments were performed in triplicate.

Manganese lixiviation of ALF472@Al-MCM-41 in PBS

2.4 mg of ALF472@Al-MCM-41 was suspended in 1 mL of PBS at 37 ºC. After incubation in darkness for 72 hours, the supernatant was collected by centrifugation using a Vivaspin® device (3500 rpm / 10 min, 30000 MWCO PES membrane). The concentration of Mn in the supernatant was determined by ICP-MS. Moreover, a similar study was performed under visible light in order to determine the lixiviation of the encapsulated metal fragments (CORMs and inactivated CORMs (iCORMs)) upon irradiation with light. Thus, suspensions of ALF472@Al-MCM-41 (concentration based on 10 μM of ALF472) were kept in PBS under visible light (using a fluorescent lamp, luminous flux: 600 lm; color temperature: 4000 K, distance between lamp and cuvette: 5 cm) at 37 °C for 72 hours. At each time, the supernatant was collected by centrifugation using a Vivaspin® device (3500 rpm / 10 min, 30000 MWCO PES membrane). The concentration of Mn in the supernatant was determined by ICP-MS.

Platinum lixiviation of ALF472-cisplatin@Al-MCM-41 in PBS

Suspensions of ALF472-*cisplatin*@Al-MCM-41 (concentration based on 10 μM of ALF472) were kept in PBS under visible light (using a fluorescent lamp, luminous flux: 600 lm; color temperature: 4000 K, distance between lamp and cuvette: 5 cm) at 37 °C for 6, 24 and 72 hours. At each time, the supernatant was collected by centrifugation using a Vivaspin® device (3500 rpm / 10 min, 30000 MWCO PES membrane). The concentration of Pt in the supernatant was determined by ICP-MS.

Characterization. N_2 adsorption isotherms were measured at 77 K on a Micromeritics Tristar 3000 volumetric instrument. Prior to measurement the powdered silica sample (Al-MCM-41)

was activated by heating at 150 °C for 24 hours and outgassing to 10^{-4} bar. The infrared spectroscopy data were collected with a Fourier transform infrared spectrophotometer Bruker Tensor 25. UV-vis spectra were collected on a Shimadzu UV Spectrophotometer. Elemental (C, H, N, S) analyses were obtained in a Flash EA1112 CHNS-O (Centre of Scientific Instrumentation of the University of Jaén). Thermogravimetric analysis were performed using a Mettler Toledo TGA/DSC STAR system under oxygen flow (20 mL min^{-1}) running from RT to 900 ºC with a heating rate of 2 °C min^{-1} (Centre of Scientific Instrumentation of the University of Granada, **Figure S1**). Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was carried out in an AGILENT 7500a (CICT, University of Jaén). Energy-dispersive X-ray Spectroscopy (EDX) analyses, mapping and TEM images were performed using a STEM PHILIPS CM20 HR microscope equipped with an EDX spectrometer operating at an accelerating voltage of 200 keV and a HAADF FEI TITAN G2 microscope also equipped with an EDX spectrometer (Centre of Scientific Instrumentation of the University of Granada). Samples were prepared by dispersing a small amount of the material (3 mg) in absolute ethanol (1 mL) followed by sonication for 10 minutes and deposition on a copper grid. DLS and *Z*-potential measurements were carried out in a Zetasizer NanoZS (Malvern) instrument of the Department of Applied Physics of the University of Granada.

RESULTS AND DISCUSSION

Synthesis and characterization of the drugs and Al-MCM-41 matrix

The cationic CORM used for our studies, ALF472 ($[Mn(tacn)(CO)_3]Br$, tacn = 1,4,7-triazacyclononane), is an air stable and water soluble manganese complex. We have recently demonstrated that this CORM is photoactivable upon irradiation with visible light, releasing 0.80

mmol of CO per mmol of complex after 24 hours, while the solutions are stable in darkness during the same timeframe.[18] In addition, the well-known anticancer drug *cisplatin*, *cis*-$[PtCl_2(NH_3)_2]$, was prepared following a previously published protocol.[30]

The porous material Al-MCM-41 was synthesized according to literature methods by doping the pristine MCM-41 matrix with $NaAlO_2$.[20] As a result, a negatively charged porous matrix (with a Si^{4+}/Al^{3+} molar ratio of 1:0.106), ready to incorporate the cationic $ALF472^+$, was obtained. The material was characterized by elemental analysis (EA), infrared spectroscopy (IR), thermogravimetric analysis (TG), N_2 adsorption at 77 K, transmission electron microscopy (TEM), and energy-dispersive X-ray spectroscopy (EDX). Thus, whilst the nitrogen adsorption/desorption isotherms confirmed the porosity of the silica material (S_{BET}: 995 $m^2\,g^{-1}$, d_{pore} (BJH): 2.97 nm, **Figure S2**), TEM images demonstrated the nanometric homogeneous size of the particles (average size: 64 ± 13 nm, **Figure S3**).

Single encapsulation of ALF472 into Al-MCM-41

With the aim of preparing new CO-releasing materials containing multiple drugs with complementary effects, the suitability of the anionic matrix Al-MCM-41 to incorporate the photoactive cationic CORM $ALF472^+$ was first evaluated. Specifically, the encapsulation process was performed by suspending, for 24 hours and in darkness, the anionic porous matrix in an aqueous solution containing the double stoichiometric amount of $ALF472^+$, with regard to the available exchangeable extra-framework cations present in the matrix. Afterwards, the suspension was filtered, washed several times with water to remove the non-encapsulated CORM, and dried at room temperature, thus producing the loaded matrix ALF472@Al-MCM-41.

Infrared spectroscopy initially confirmed the successful incorporation of the CO-prodrug within the porous nanomaterial (**Figure 1**). Indeed, the characteristic CO stretching bands of ALF472 appear slightly shifted in the hybrid material compared to pristine CORM (from υ = 2017 cm^{-1} to 2029 cm^{-1} and from υ = 1895 cm^{-1} to 1927 cm^{-1}), as has been previously observed for other related materials and attributed to the confinement of CORM molecules within the pores.[18,20,21]

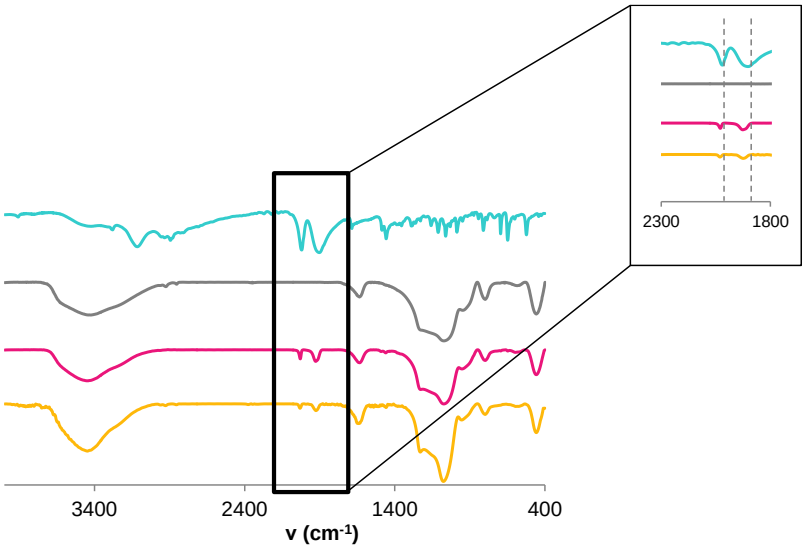


Figure 1. Infrared spectra of free ALF472 (turquoise line), mesoporous silica Al-MCM-41 (grey line) and hybrid materials ALF472@Al-MCM-41 (pink line) and ALF472-*cisplatin*@Al-MCM-41 (yellow line). The displacement of the CO stretching bands in the hybrid materials in comparison to the free CORM is highlighted.

In addition, the amount of encapsulated $ALF472^{+}$ was quantified by a combination of techniques (namely, EDX, elemental and thermogravimetric analysis). The results revealed that the aluminium doped MCM-41 shows a better performance in terms of loading (0.48 mmol $[ALF472]^{+}$ g^{-1} dried basis) than the previously reported sulphonic-functionalized MCM-41 (0.22 mmol $[ALF472]^{+}$ g^{-1} dried basis).[18] This improvement can be explained taking into account the

presence of a higher amount of anionic exchangeable sites in Al-MCM-41 in comparison to MCM-41-SO_3H (1.2 mmol g^{-1} and 0.22 mmol g^{-1}, respectively).

Moreover, while the previously reported ALF472@MCM-41-SO_3 hybrid material was obtained as particles in the micrometer range, ALF472@Al-MCM-41 was isolated as nanoparticles. Indeed, TEM images revealed that the loaded material showed a similar size (49 ± 11 nm, **Figure S4**) and morphology to the empty matrix (64 ± 13 nm). In fact, the nanometric nature of ALF472@Al-MCM-41 may add further advantages to this new hybrid material in terms of its administration through different routes (i.e. intravenous). Regarding hydrodynamic size and *Z*-potential values, both parameters confirmed the good dispersion of empty Al-MCM-41 (93 ± 28 nm and - 28 ± 1 mV) and loaded ALF472@Al-MCM-41 (112 ± 33 nm and -23 ± 1 mV) in simulated biological conditions. Finally, EDX elemental mapping of ALF472@Al-MCM-41 nanoparticles corroborated the presence of homogeneously distributed manganese, silicon and aluminium as well as the absence of bromide (**Figure S5**). These results suggest that $ALF472^+$ is encapsulated into the pores of the matrix as a consequence of an ion exchange process and not merely co-precipitated.

Dual encapsulation of ALF472 and cisplatin into Al-MCM-41

Taking into account the successful incorporation of ALF472 into the Al-MCM-41 nanoparticles, we decided to further explore the ability of this matrix to encapsulate multiple drugs, which could exert a synergistic or complementary therapeutic effect.[26–28] As a proof of concept, we carried out, for the first time, the dual encapsulation of our photoCORM, ALF472, and the well-known antitumoral agent *cisplatin*. The loading was performed by one pot solid-liquid impregnation process in water and in the absence of light in order to avoid the photoactivation of

the CORM. Once again, the successful incorporation of $ALF472^+$ in the matrix was first confirmed by infrared spectroscopy, showing the characteristic displacement of CO stretching bands due to the confinement of the CORM into the cavities of Al-MCM-41 (υ = 2031 cm^{-1} and 1925 cm^{-1} for ALF472-*cisplatin*@Al-MCM-41) (**Figure 1**). The cargo of both drugs was quantified by elemental and thermogravimetric analysis as well as EDX, yielding 0.45 mmol g^{-1} of $ALF472^+$ and 0.12 mmol g^{-1} of the platinum complex. Regarding the loading of $ALF472^+$, the amount of encapsulated CORM was similar to that of ALF472@Al-MCM-41 (0.48 mmol g^{-1}), confirming that *cisplatin* does not hamper the cation exchange encapsulation process in the pores of Al-MCM-41. In contrast, the single loading of *cisplatin* into the matrix substantially decreased its cargo threefold (0.038 mmol g^{-1}). This result may suggest that the presence of $ALF472^+$ favours the loading of the anticancer drug probably due to the formation of hydrogen bonds between both guest molecules. Indeed, a similar trend has been previously reported by Delgado *et al*. when using MCM-41 and MCM-41-NH_2 as hosts for the encapsulation of *cisplatin*.[33] In fact, the amino derivatized silica, ready to establish hydrogen bonds, incorporated 0.204 mmol g^{-1} of *cisplatin* while the non-functionalized matrix only loaded 0.029 mmol of this metallodrug per gram.

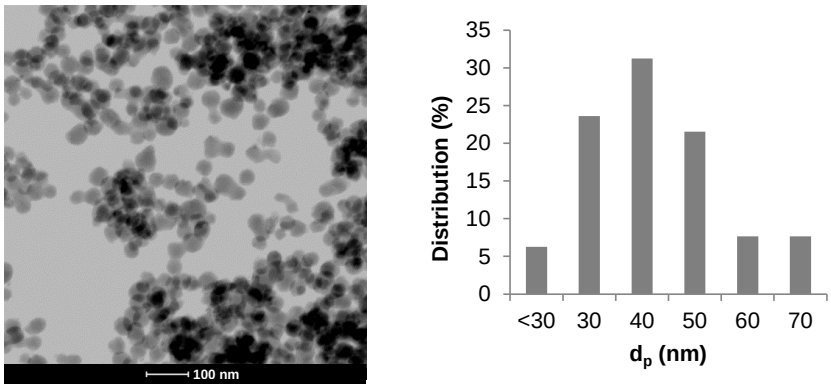


Figure 2. TEM image of ALF472-*cisplatin*@Al-MCM-41 nanoparticles and size distribution profile (average size 48 ± 14 nm), n > 100.

Moreover, TEM images confirmed that the dual encapsulation process did not alter either the morphology or size of the loaded nanoparticles (48 ± 14 nm, **Figure 2**), although a small aggregation takes place when particles are suspended in physiological medium (hydrodynamic size: 336 ± 50 nm, *Z*-potential: - 21 ± 1 mV). A similar trend was also observed for *cisplatin*@Al-MCM-41 (hydrodynamic size: 207 ± 23 nm, *Z*-potential: - 29 ± 1 mV). In spite of this aggregation effect, particle size is still appropriate for a potential biological application, as it has been demonstrated that silica nanoparticles ranging from 50 to 300 nm can be easily internalized by endocytosis without any significant toxicity effects.[34] Finally, elemental mapping obtained by EDX further confirmed a homogeneous distribution of both drugs in Al-MCM-41 nanoparticles (**Figure 3**) as well as the absence of bromide counter ions (**Figure S6**).

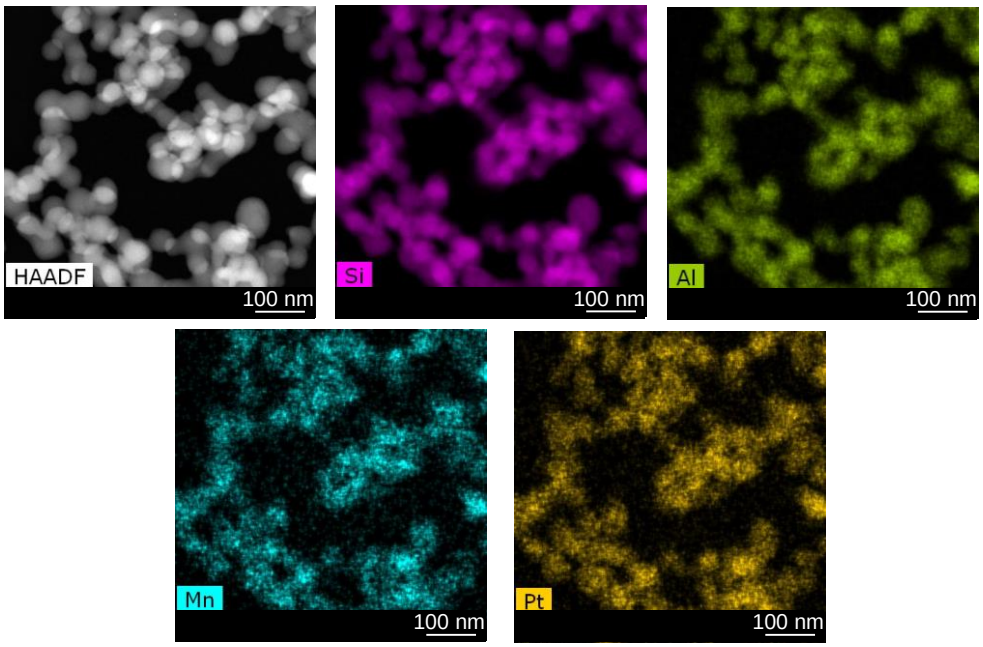


Figure 3. Elemental mapping obtained by EDX of the dual hybrid material ALF472-*cisplatin*@Al-MCM-41. The presence of silicon, aluminium, manganese and platinum elements into the nanoparticles is confirmed.

CO delivery studies of the loaded materials

In order to envisage a potential biological application as CORMAs of ALF472@Al-MCM-41 and the dual hybrid system ALF472-*cisplatin*@Al-MCM-41, the photo induced CO release properties of these materials were investigated in simulated biological conditions (10 mM PBS, 37 °C) by UV-vis spectroscopy using the myoglobin assay. For this purpose, a degassed PBS solution of equine heart myoglobin was freshly reduced with excess of sodium dithionite under inert atmosphere and then the loaded matrix under study was added. As shown in **Figure 4**, the CO release kinetic profile of ALF472@Al-MCM-41 is quite different from that of the free CORM. Indeed, a less steep slope is observed for the encapsulated CO-prodrug ($k_{\text{ALF472@Al-MCM-41}}$: 0.017 min^{-1}), which may allow a more controlled CO supply than that of free CORM (k_{ALF472}: 0.031 min^{-1}). Moreover, the total amount of released CO after 24 h, accounts for 0.25 mmol of CO per mmol of encapsulated $ALF472^{+}$, while free CORM delivers 0.80 mmol of CO per mmol of complex in the same timeframe. Regarding the dual material, ALF472-*cisplatin*@Al-MCM-41 shows a similar CO releasing rate than ALF472@Al-MCM-41 ($k_{\text{ALF472-}cisplatin\text{@Al-MCM-41}}$: 0.017 min^{-1}), although it delivers 0.11 mmol of CO per mmol of CORM after 24 hours, which accounts approximately for the 50% of the total amount of CO released from the single material ALF472@Al-MCM-41. Hence, the lower capacity of ALF472-*cisplatin*@Al-MCM-41 to deliver CO upon activation with visible light may be related to the stabilization of CO ligands due to the formation of H-bonds involving both guest molecules.

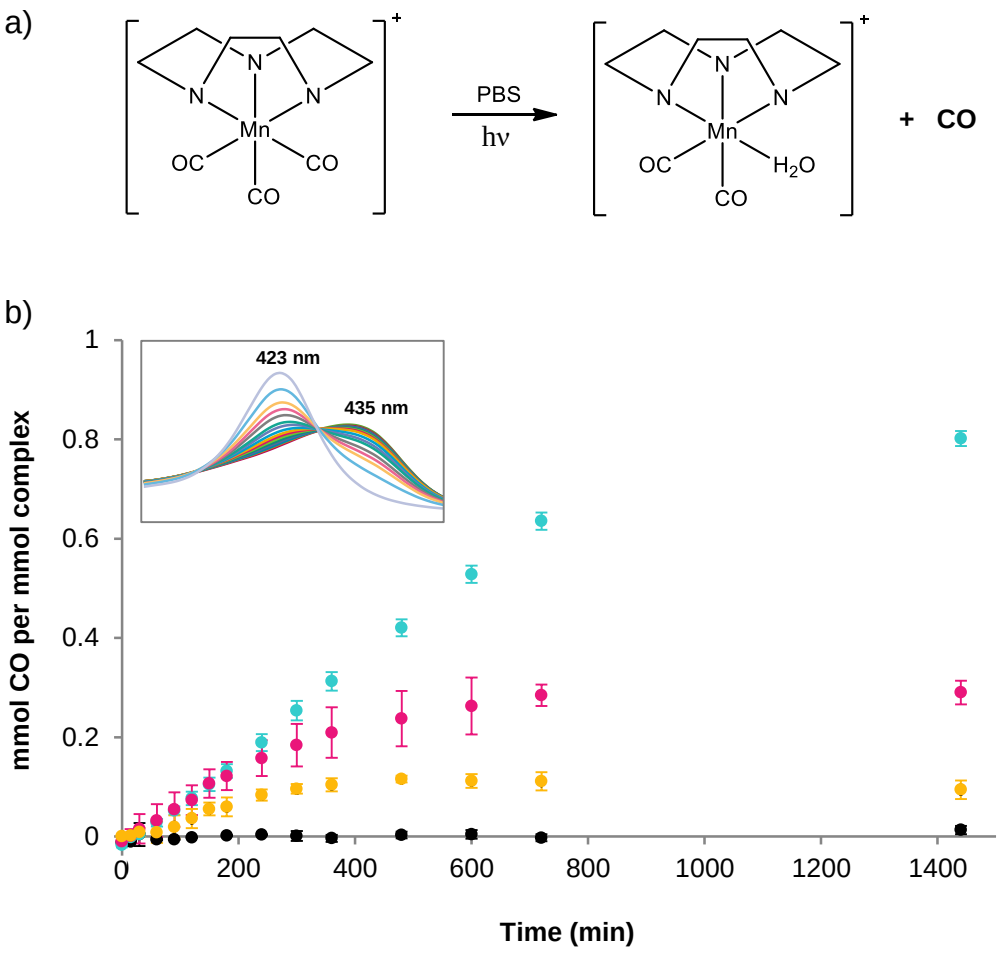


Figure 4. a) Photoactivation process of $[Mn(tacn)(CO)_3]^+$ ($ALF472^+$) under simulated physiological conditions. b) CO releasing kinetics upon irradiation with visible light of the free complex $ALF472^+$ (turquoise circles) and the hybrid materials ALF472@Al-MCM-41 (pink circles) and ALF472-*cisplatin*@Al-MCM-41 (yellow circles). Black circles demonstrate that there is no CO release from ALF472 in darkness. The inset shows the evolution of the electronic spectra of myoglobin upon CO coordination. Experimental conditions: Ar atmosphere, 10 mM PBS, 37 ºC, 10 µM of ALF472 and 10 µM of myoglobin.

Metal leaching studies of the hybrid materials

With the aim of analyzing the ability of Al-MCM-41 to retain $ALF472^+$ inside its channels, we then evaluated the manganese leaching of a suspension of the hybrid material in saline phosphate buffer (PBS) in darkness. In fact, the presence of high concentrations of salts in the buffer could promote cationic-exchange processes with the subsequent desorption of $ALF472^+$ to the media. Thus, the results revealed that the porous matrix Al-MCM-41 exhibited a better performance towards the retention of $ALF472^+$ compared to the previously reported MCM-41-SO_3H. Indeed, the aluminium doped silica matrix is able to keep ca. 86 % of CORM trapped after 72 hours of incubation in darkness, whilst the corresponding alkylsulphonic matrix only retained the 14 % of $ALF472^+$ after 1 hour.[18] These results suggest that the interaction between $ALF472^+$ and the aluminium doped host is stronger than that established with the alkylsulphonic functionalized one, probably due to the different location and distribution of the anionic sites within the matrix. Indeed, in the case of MCM-41-SO_3H, the sulphonic groups might be mainly located in the surface of the material while in the case of Al-MCM-41 the substitution of Si^{4+} by Al^{3+} is likely to occur along the framework with a more homogeneous distribution. Regarding the metal leaching upon irradiation with visible light, a similar manganese leaching from ALF472@Al-MCM-41 was observed (20 % after 72 hours), which again confirm the high ability of the doped matrix to keep trapped not only $ALF472^+$ but also the corresponding decarbonylation fragments produced after CO release.

After proving the good retention capacity of Al-MCM-41 towards the potentially toxic manganese fragments, we quantified the delivery of *cisplatin* from the dual material in the same experimental conditions used for monitoring CO delivery (PBS, light). The results demonstrated that, approximately, 5% of Pt is leached to the physiological medium after 6 hours, and no

significant increase was observed during the next 66 hours (total amount of leached Pt at 72 hours: 7%). Hence, the high retention of this anti-cancer drug within the cavities of the anionic silica framework may be related to several issues, such as the formation of hydrogen bonds between *cisplatin* and CORM molecules as well as the generation of platinum cationic species due to the exchange of chlorine ligands by water molecules.

CONCLUSIONS

In this study, we have shown that it is possible to prepare new CORMAs based on the cationic photoCORM ALF472^{+} [Mn(tacn)(CO)$_3$]$^{+}$, (tacn =1,4,7-triazacyclonane) and the biocompatible nanometric mesoporous silica Al-MCM-41, by means of a simple ionic exchange strategy. In addition, we have proved the successful incorporation of two complementary drugs, ALF472^{+} and *cisplatin*, withinthe cavities of this silica matrix. Neither encapsulation process altered the size or the morphology of the nanoparticles. It is worth noting that the presence of CORM molecules increase significantly the cargo of the platinum anticancer drug suggesting a synergistic loading effect. In addition, both hybrid materials show a more controlled CO release than the free CORM, efficiently trapping most of the metal fragments, both loaded and generated. In fact, it should be highlighted that the host-guest interactions are strong enough to prevent the exchange of the CORM with the ions present in the physiological medium as well as avoiding the leaching of the potentially toxic decarbonylation fragments. Although the dual hybrid material does exhibit a low release of *cisplatin*, it may be considered as a proof of concept of the feasibility of developing CORMAs with multiple therapeutic effects.

ASSOCIATED CONTENT

Supporting Information: Thermal analysis, nitrogen adsorption isotherms, EDX analysis, TEM images and size distribution profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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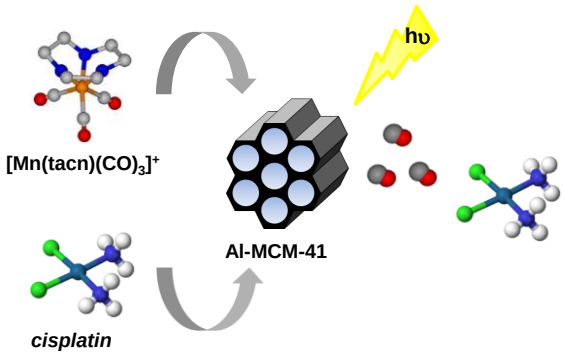
Table of Contents, Graphic and Synopsis



Aluminium mesoporous silica nanoparticles, simultaneously hosting a manganese photoCORM and *cisplatin*, deliver CO in a controlled manner upon irradiation with light, whilst keeping most of the potentially toxic photoproducts trapped.