CrystEngComm

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *CrystEngComm*, 2013, **15**, 9364

Received 1st July 2013,
Accepted 3rd September 2013

DOI: 10.1039/c3ce41289j

www.rsc.org/crystengcomm

Metal–organic frameworks as potential multi-carriers of drugs†

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The metal–organic framework CPO-27-Ni is presented as a proof-of-concept model for the incorporation and release of two non-conventional anticancer drugs: [Ru(*p*-cymene)Cl_2(pta)] (RAPTA-C) and NO.

Nowadays, one of the most important challenges in our society concerns the development of a more effective cancer treatment with reduced secondary effects. Indeed, the effectiveness of drugs in cancer therapy is mainly limited by their inadequate transport within the body and unspecific delivery to the cancer cells. Tumors have tortuous and defective vasculatures, with substantial heterogeneity, and, therefore, the net effects are both an increased flow resistance and hypoxia, which are major obstacles in cancer therapy.[1]

In this regard, metal–organic frameworks (MOFs),[2] which are a new class of synthetic, porous materials, with exceptionally high adsorption performances, have recently been studied as selective drug carriers.[3] It is noteworthy that some of these materials exhibit a dual hydrophilic/hydrophobic pore structure, as well as an unusually high concentration of exposed coordinatively unsaturated metal sites, which make them suitable as multicarrier delivering agents. In this regard, the simultaneous adsorption and delivery of a wide range of probe molecules, such as bioactive gases (*e.g.* NO and CO)[4] or small organic drugs (*e.g.* busulfan and cidofovir),[5] might be possible.

It has been proven that nitric oxide (NO) is an important species involved in many biological processes.[6] Indeed, it mediates a number of vital functions, including vasodilatation[7] and re-epithelization.[8] The accumulation of high concentrations of nitric oxide in tumours offers the possibility to increase cancer cells' sensitivity to chemo- and radiotherapy or even to suddenly induce their apoptosis.[9] Remarkably, recent studies described the use of NO to enhance cisplatin cytotoxicity in *V79* lung fibroblast cells taking advantage from the direct sensitizing of tumour cells.[10]

A number of porous materials have been described as delivering agents for exogenous NO. This is the case for some zeolites[11] and the $[M_2(C_8H_2O_6)(H_2O)_2]\cdot(H_2O)_8$ (CPO-27-M) coordination polymer series (M = Ni, Zn, Mg, Mn, Fe, and Co; $C_8H_2O_6$ = 2,5-dihydroxyterephthalic acid). Regarding the latter, the first highly crystalline member of the series based on Co^{2+} cations (CPO-27-Co) was synthesized by Dietzel and co-workers in 2005.[12] Since this initial synthesis, several analogues have been produced containing Ni^{2+},[13] Zn^{2+},[14] Mg^{2+},[15] Mn^{2+}[16] and very recently Fe^{2+}.[17] Particularly, the Ni^{2+} derivative presents a high adsorption–delivery capacity for NO.[18] The presence of coordinatively unsaturated metal sites (CUSs) on the pore walls has a clear effect on the NO adsorption and release processes. Indeed, during the adsorption process, an adduct Ni–NO is formed which makes possible the incorporation and posterior water-triggered release of up to 7 mmol of NO per g of material. Furthermore, it has been shown that CPO-27-Ni shows a reasonable balance between stability and solubility in bovine serum after an extended period, which is a key issue in biological applications.[19]

Therefore, taking into account the exceptional reversibility of the NO adsorption in CPO-27-Ni and its stability in biological conditions, we have studied whether this material can act as a vehicle for multiple drugs, namely, [Ru(*p*-cymene)Cl_2(pta)] (pta = 1,3,5-triaza-7-phosphaadamantane) (RAPTA-C) and NO. This organometallic ruthenium(II) arene complex has been chosen as it is a non-conventional metallodrug showing significant *in vivo* antitumor activity towards lung metastases in mice.[20]

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† Electronic supplementary information (ESI) available: Details of experimental procedures, chemical stability tests, elemental analysis, IR spectra and thermal analysis. See DOI: 10.1039/c3ce41289j

In addition, some of us have previously reported that the incorporation/release of RAPTA-C in the highly porous and robust $[Ni_8(OH)_4(OH_2)_2(4,4'\text{-(buta-1,3-diyne-1,4-diyl)bispyrazolato})_6]_n$ MOF is possible.[21] In this communication, we report on the incorporation of the non-conventional RAPTA-C metallodrug into the free porous space of CPO-27-Ni and the subsequent uptaking of NO (Fig. 1). Then, the release of the loaded bioactive molecules in SBF has also been successfully achieved. The results are a proof of concept of the possibility to load and deliver multiple drugs in MOFs for the development of synergic anticancer therapies with enhanced effectiveness.

Samples of CPO-27-Ni[13] and RAPTA-C[22] were prepared as described in the literature (for experimental details, see the ESI†). Thermal activation of CPO-27-Ni at 423 K removes the water guest molecules, leaving open metal sites ready for NO adsorption. It has been proven that the activated CPO-27-Ni possesses remarkably high chemical stability, which is exemplified by its unchanged powder X-ray diffraction (XRPD) pattern upon stirring for 72 h in anhydrous methanol, tetrahydrofuran and dichloromethane (Fig. S1†). This property let us perform the incorporation of RAPTA-C into the porous matrix by suspending it in an 80% saturated solution of RAPTA-C. The loading of the drug to give CPO-27-Ni@RAPTA was preferentially achieved in CH_2Cl_2 as this solvent led to the highest uptake accounting for ~4 RAPTA-C molecules per unit cell as calculated using elemental analysis and UV–vis (see ESI†). This result is probably due to the major concentration of RAPTA-C in the impregnating solution as the solubility of RAPTA-C in the apolar CH_2Cl_2 is significantly higher than in MeOH or tetrahydrofuran. XRPD of the CPO-27-Ni@RAPTA system reveals that the crystallinity of the original porous matrix is retained (Fig. S2†), indicating that the porous matrix has not changed during the loading process. Furthermore, the actual incorporation of the Ru-metallodrug is proven by i) the dramatic reduction of the adsorption capacity of the MOF (SBET drops from 720 $m^2\ g^{-1}$ for the original material to 45 $m^2\ g^{-1}$ after RAPTA-C loading) (Fig. 2a); ii) the presence of the main peaks of the pure RAPTA-C in the IR spectrum of CPO-27-Ni@RAPTA (Fig. S3†); and iii) the increase of the thermal stability of CPO-27-Ni@RAPTA compared to the unloaded CPO-27-Ni material, together with a less steep weight loss for the decomposition process (Fig. S4†).

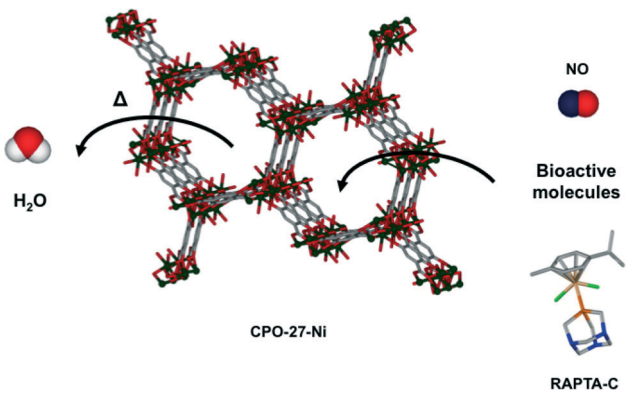

Fig. 1 Thermal activation of the CPO-27-Ni metal–organic framework to remove the solvent guest molecules and the subsequent loading with NO and RAPTA-C. Color code: H: white, C: grey, O: red, N: blue, Ni: dark green, Ru: light orange, P: orange, Cl: bright green.

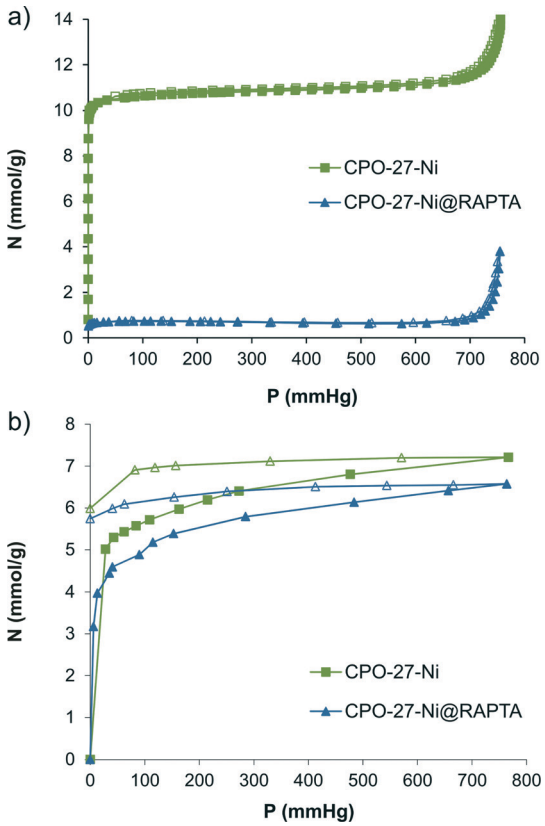

Fig. 2 a) N_2 (77 K) and b) NO (298 K) adsorption isotherms for activated CPO-27-Ni (green squares) and the loaded CPO-27-Ni@RAPTA-C derivative (blue triangles). Empty symbols denote desorption.

Once the porous matrix was loaded with the non-conventional metallodrug RAPTA-C, gravimetric NO adsorption measurements at room temperature revealed that CPO-27-Ni@RAPTA adsorbs 6.0 mmol of NO per g of activated material (Fig. 2b). This value is very close to the one obtained for the original CPO-27-Ni (7 mmol NO per g of MOF), which means that the presence of RAPTA-C in the cavities does not hamper the access of NO to the open metal sites. The adsorbed NO accounts for ~1 NO molecule per CUS, although there will also be a small amount of weakly physisorbed NO interacting with the pore surface. Moreover, the shape of the NO isotherms is maintained showing a hysteresis loop typical of those materials in which the NO molecule strongly binds the metal atom. This fact supports that the main NO incorporation mechanism (chemisorption) is independent from that of the RAPTA-C adsorption process (physisorption). The presence of NO in the porous matrix was confirmed with IR spectroscopy (Fig. S5†). The stretching band at 1839 cm^{-1} is somewhat less intense than the one found for the original CPO-27-Ni material loaded with NO, which further supports the lower amount of adsorbed NO in

comparison with the original porous matrix, as a result of the space occupied by the RAPTA-C molecules inside the cavities. Similar shape of the band and its position confirms adsorption on the CUS sites inside the channels of the material and not only physisorption at the external surface.

As previously reported,[18] water molecules can trigger the delivery of coordinated NO, replacing them from the open metal sites. Fig. 3 shows the releasing profile of NO for CPO-27-Ni@NO and CPO-27-Ni@RAPTA-C@NO when exposed to a wet gas containing 11% of relative humidity. As previously reported for CPO-27-Ni, CPO-27-Ni@RAPTA also releases physisorbed NO very quickly as a sharp spike appearing within the first few minutes of the kinetic of release (Fig. 3). However, the release of the chemisorbed NO is much slower and seems to be affected by RAPTA-C inclusion into the pores. Indeed, CPO-27-Ni@RAPTA-C@NO releases NO significantly faster (half life of release = 23 min) than unloaded CPO-27-Ni@NO (half life of release = 40 min), as appreciated by a steeper drop of NO concentration for the former material. The total amount of stored NO is completely released in both cases. Although in the case of CPO-27-Ni@RAPTA-C@NO, the major quantity of stored gas is recovered within 2 hours, after 12 hours, a significant amount of NO is still being released. These results indicate that the incorporation of RAPTA-C into the pores of the framework does not significantly affect the NO adsorption/desorption processes. Nevertheless, we can appreciate a change in the desorption kinetics, which is faster in the case of the RAPTA-C loaded material.

The further step has been the study of the RAPTA-C delivery process from the CPO-27-Ni@RAPTA-C@NO loaded material and the evaluation of the effects of NO incorporation. For this purpose, we suspended two samples of CPO-27-Ni@RAPTA-C, untreated and NO loaded, into a simulated body fluid (SBF) at 310 K in order to compare RAPTA-C desorption kinetics profiles. As depicted in Fig. 4, there are no significant differences for the NO loaded and unloaded samples, since both samples release about 25% loaded RAPTA-C within 2 h. This behaviour is

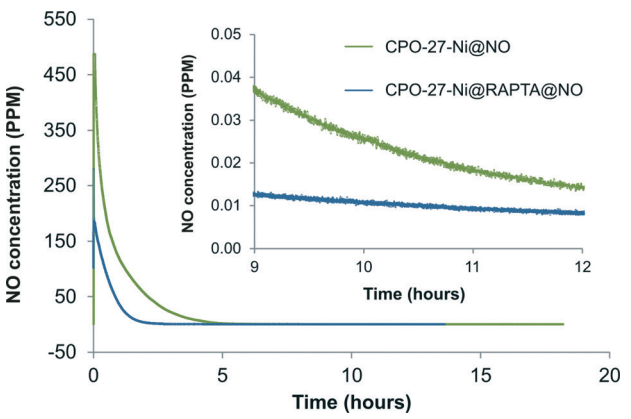

Fig. 3 Delivery of NO on contact with wet gas (11% relative humidity), as measured by chemiluminescence. The materials are still releasing NO at and beyond 12 h under these conditions.

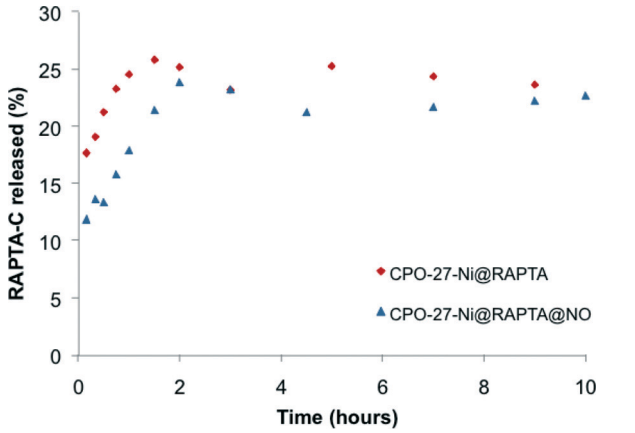

Fig. 4 Desorption kinetics profile of RAPTA-C in SBF at 310 K from the original CPO-27-Ni porous matrix and the one loaded with NO. Both materials behave in a similar manner realising the 25% of RAPTA-C in 2 h.

reasonable as no interactions between adsorbed RAPTA-C and NO take place. Indeed, it is known that the delivery rate of NO from CPO-27-Ni is increased markedly in physiological solutions with the half-life of delivery being reduced from several hours for flowing gas to a few minutes in contact with PBS.[18] Then, considering that the NO release from CPO-27-Ni@RAPTA@NO into SBF is nearly instantaneous, the porous matrix becomes essentially identical to the unloaded CPO-27-Ni@RAPTA, which further supports the results. It should be noted that the prompt release of NO may have a beneficial impact in biological applications due to the vasodilation properties of this signalling molecule, which may help the penetration of RAPTA-C into the cancer cells.

Conclusions

In this study, we proved that the concomitant adsorption of nitric oxide and RAPTA-C into the pores of CPO-27-Ni material is feasible. The adsorption capacity for both species is basically unaffected by the presence of each other. This is attributable to the different interactions taking place between the framework walls and the adsorbate of interest: NO directly interacts with the Ni open metal sites (chemisorption) while RAPTA-C is only physisorbed into the pores. On the other hand, the release of the trapped Ru-metallodrug into SBF does not depend on the previous loading of NO into the framework, substantially, because of the immediate release of NO in an aqueous medium. However, the kinetics of NO desorption in the presence of a humid flowing gas is significantly faster for the CPO-27-Ni@RAPTA-C than for the original CPO-27-Ni.

The investigated system must be considered as a proof of concept of the feasibility of the concurrent adsorption of bioactive molecules into the metal–organic frameworks. Further investigation is envisaged for the development of novel systems in which the concomitant incorporation of two (or more) bioactive species results in the neat improvement of the

delivery performances. It should be noted that the exploitation of the synergic effect of multiple drugs may lead to advanced combined therapies.

Acknowledgements

The authors are grateful for the generous support by the Spanish MCINN (project: CTQ2011-22787 and E. Q.-P. FPI fellowship), Junta de Andalucía (project: P09-FQM-4981 and S. R. pre-doctoral fellowship) and COST Action CM1105. R. E. M. thanks the EPSRC (grant numbers EP/K025112/1 and EP/K005499/1) and the Royal Society for provision of an Industry Fellowship. The IR studies (B. G. and B. M.) were carried out with the equipment purchased thanks to the financial support of the European Regional Development Fund in the framework of the Polish Innovation Economy Operational Program (contract no. POIG.02.01.00-12-023/08).

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