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Giada Mannias,[a,b] Alessandra Scano,[a*] Martina Pilloni,[a] Edmond Magner[b] and Guido Ennas[a*]

[a]Department of Chemical and Geological Sciences, University of Cagliari and INSTM unit, S.S. 554 bivio Sestu, 09042 Monserrato (CA), Italy;

[b]Department of Chemical Sciences and Bernal Institute, University of Limerick, Limerick, V94 T9PX, Ireland.

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Tailoring MOFs to biomedical applications: a chimera or a concrete reality? The case study of Fe-BTC by bio-friendly Mechanosynthesis

Giada Mannias,[a,b] Alessandra Scano,[a]* Martina Pilloni,[a] Edmond Magner[b] and Guido Ennas[a]*

[a]Department of Chemical and Geological Sciences, University of Cagliari and INSTM unit, S.S. 554 bivio Sestu, 09042 Monserrato (CA), Italy; [b]Department of Chemical Sciences and Bernal Institute, University of Limerick, Limerick, V94 T9PX, Ireland.

*E-mail: alescano80@tiscali.it, ennas@unica.it

Tailoring MOFs to biomedical applications: a chimera or a concrete reality? The case study of Fe-BTC by bio-friendly Mechanosynthesis

We point-out an exceptional tailorability of iron(III) trimesate metal-organic framework to bio-friendly conditions. This is particularly attractive in sight of one-pot immobilization of biomolecules for biomedical applications. Synthesis is carried out via mechanochemical approach under green, biocompatible conditions without additional solvents, in just 1 hour, at room temperature. Solvents are proven to be unnecessary to build the framework, in contrast to solution-based methods. Microstructure and thermal stability of the material are not affected by pH. Conversely, textural properties can be tuned by simply varying the amount of base.

Keywords: iron(III) trimesate; metal-organic framework tailorability; mechanochemistry; green chemistry; biocompatibility

1. Introduction

Metal-organic framework (MOF) materials are porous coordination polymers consisting of metal centers, also called nodes, joined together by organic linkers through coordination bonds to build one-, two- or three-dimensional architectures.[1] Several metal ions and organic ligands can be combined to form a range of very different structures, proving a remarkable chemical variability of MOFs. Metal-organic frameworks are commonly used as supports for biomolecule immobilization.[2] Compared to mesoporous silica, MOFs lead to lower levels of leaching from the supports.[3] This is because of the strong interactions between the organic components of the framework and the protein molecules (e.g. π-π interactions).[4] To the best of our knowledge, a wide range of proteins and enzymes has been immobilized on MOFs to develop drug-delivery systems, biosensors, and biocatalysts for applications in numerous fields, including food and environment safety, and biomedicine.[5–14] This is for example the case of cholesterol oxidase (ChOx) for cholesterol detention,[15] carbonic anhydrase (CA) to promote CO_2 capture,[16] insulin as drug-delivery system,[17] and so on. Unlike free proteins, their immobilization on supports provides a heterogeneous environment for facile separation and reuse, enhanced stability and substrate selectivity.[18]. Besides enzymes, and more generally proteins, MOFs have been investigated as protective matrix for a range of bioentities, including nucleic acids (DNA[19] and RNA[20]), antibodies,[21] drugs,[22] cells and viruses.[23] Immobilization strategies could involve post-synthetical approaches, which firstly need prefabrication of the support and a consequent attachment/incorporation of the biomolecules via adsorption or covalent linkage. Such approaches give mostly rise to a superficial immobilization of the guests, which result still affected by the external environment.[3] On the other hand, *in situ* (one-pot) encapsulation results in the direct incorporation of the guest molecules into the support framework during synthesis.[24] It has become very attractive since no additional steps are needed, minimizing production times, costs, and environmental footprint. Moreover, it favors the immobilization of molecules larger than the pore size of the support and prevents the leakage from support.[25] One-pot immobilization requires preserving the chemical-physical integrity of the support, while efficiently shielding the guest molecule from the external environment, improving its overall stability and retaining its functionality[26]. Zeolitic imidazolate frameworks (ZIFs) are the most common MOFs used for *in situ* immobilization of biomolecules owing to their extremely mild synthetic conditions.[21,27–29] However, when the synthesis of the support is not performed in aqueous media under mild pH and temperature, post-synthetical immobilization strategies are inevitably required.[26] Additional steps are involved, resulting in higher costs and the use of more reagents.[25] Consistently, trying to bring MOFs' harsh synthesis conditions closer to those required to avoid the biomolecule denaturalization would fully display their application spectrum, resulting in sustainable, time- and cost-efficient *in situ* immobilization strategies which avoid the above-mentioned drawbacks of post-synthesis approaches. However, tailoring of the synthetic conditions of the support to bio-friendly levels without compromising its structural and textural features can be very challenging. For instance, post-synthetical encapsulation strategies are often required for iron(III) trimesate MOFs,[22,30–33] since their traditional harsh synthesis conditions far from biological pH levels would prevent the retention of guest's biological activity.[34] Therefore, applications of these MOFs as biomolecules support are often hindered, despite the great potential offered by such framework. Indeed, iron trimesate MOFs, assembled from iron(III) and 1,3,5-benzenetricarboxilate (BTC or trimesate), have attracted enormous attention because of their high specific surface areas (up to 2800 m^2/g), high biocompatibility, lack of *in vivo* toxicity, low cost and stability in air, water and organic solvents.[35–37] Moreover, they can undergo redox processes. It has been demonstrated that the partial and controlled reduction of Fe(III) to Fe(II) sites in the MOF upon heating under vacuum leads to coordinatively unsaturated metal sites (CUS), which exhibit strong interactions with unsaturated molecules (e.g. CO, NO and propylene) via π-back bonding.[38] Iron(III) trimesate MOFs also possess intrinsic peroxidase-like activity, catalyzing the oxidation of peroxidase substrates in the presence of H_2O_2.[39] These features make such MOFs interesting candidate materials for applications in areas including catalysis,[40,41] energy storage,[42] degradation of organic pollutants,[43,44] gas storage and separation,[45,46] sensing, [47,48] drug-delivery[32,33] and protein immobilization.[49,50] MIL-100(Fe) (MIL: Materials Institute Lavoisier) was the first iron(III) trimesate MOF to be synthetized.[51] It is a highly crystalline network comprised of trimers of iron octahedra sharing an μ_3-oxo anion as common vertex. These trimeric secondary building units (SBUs) are connected through 1,3,5-benzenetricarboxylate moieties, giving rise to hybrid supertetrahedra that in turn constitute a three-dimensional zeolitic architecture of MTN type (MTN: Mobil Thirty-Nine). This structural topology delimits two types of mesoporous cages with diameters of ca. 25 and 29 Å, accessible through pentagonal and hexagonal microporous windows with free diameter of ca. 5.5 and 8.6 Å, respectively. Traditional synthesis of this system is based on solvo/hydrothermal method which requires long reaction times (6 days), high costs, the use of large amounts of solvents and reagents (e.g. HF), as well as high pressure and temperature (150 °C) and acidic conditions ($pH < 1$).[51] Efforts have been made to prepare the material without fluorine via solvo/hydrothermal routes.[52,53] More recently, Sanchez-Sanchez et al. have developed a solution-based method for the synthesis of MIL-100(Fe) at room temperature.[54] However, long reaction times (24 hours) and the use of NaOH, which is strongly irritating and corrosive, are needed. Milder and greener synthetic conditions have been achieved by exploiting alternative methods (e.g. microwave[55], mechanochemistry[56] and sonochemistry[57]). These methods use smaller amounts of solvents, are cost-efficient and faster than traditional ones, giving rise to the formation of Fe-BTC, the disordered phase of MIL-100(Fe).[58] Fe-BTC materials show comparable features with MIL-100(Fe), sometimes performing better than the crystalline counterpart (e.g. Lewis acid reactions).[59] They are commercially available (e.g. Basolite F300 produced by BASF)[60] and are usually used for same applications.[61] Mechanochemistry also offers an opportunity to overcome the limits given by solution-based harsh conditions, because it uses a trace amount or no solvents, minimizing biomolecules exposure to the reaction bulk. Recently, mechanochemical approaches have been widely employed to synthesize MOFs, using small (LAG - Liquid-Assisted Grinding) or no additional solvents (NG – Neat Grinding).[34,62] These approaches lead to high yields in short reaction time under mild conditions, and can be easily scaled up.[63] Moreover, it has been observed that biomolecules are generally more stable in powder than in solution.[64] For example, *in situ* enzyme immobilization on UiO-66-NH_2 (UiO: Universitetet i Oslo) was performed for the first time by Wei et al. using a two-step mechanochemical synthesis.[34] First, MOF precursors were grinded with a little amount of ethanol (LAG). Once MOF seeds were formed, enzyme powder (β-glucosidase or invertase) and more MOF precursors were added without additional solvents. Such an approach was extended to ZIF-8 and Zn-MOF-74 for the *in situ* immobilization of catalase via ball milling.[34]

Regarding iron(III) trimesate materials, only solution-based processes have been reported so far to accomplish *in situ* enzyme encapsulation.[50,65–69] Gascon et al. integrated alcohol dehydrogenase (ADH), glucose oxidase (GOx) and lipase B (Lip) during the synthesis of Fe-BTC.[65,66,69] An aqueous solution of enzyme was added to a trimesic acid containing NaOH solution. Then, an aqueous solution of ferric chloride was dropwise added. The resulting suspension was kept under magnetic stirring for 10 minutes at room temperature. An analogous protocol was followed by Tocco et al. to encapsulate laccase (LAC) on Fe-BTC and ZIF-zni, finding much lower laccase activity for Fe-BTC based biocatalyst compared to ZIF-zni.[68] Zhao et al. immobilized GOx on Fe-BTC via one-pot solution-based synthesis.[67] An ethanolic solution of trimesic acid was added to an aqueous solution containing GOx and polyvinylpyrrolidone (PVP). Then, an aqueous solution of ferric chloride was slowly added and kept under stirring for 30 minutes. Such hybrid composite was applied as biosensor for glucose colorimetric detection, using a cascade reaction which involves the catalytic activity of GOx and the peroxidase-mimic activity of Fe-BTC finally producing the oxidation of a chromogenic substrate (e.g. 3,3’,5,5’-tetramethylbenzidine, TMB). Analogously, synergistic enzymatic and enzyme-mimicking activity of a catalase-integrated Fe-BTC material was used for the colorimetric sensing of hydrogen peroxide and phenol by Jing et al.[50] An ethanolic solution of trimesic acid was added to an aqueous solution containing the enzyme. Then, an aqueous solution of ferric nitrate was added, and the resulting suspension was kept under stirring for 4 hours. All the above-mentioned protocols require relatively short reaction times (from 10 minutes to 4 hours), ambient temperature and pressure conditions. However, enzymes result expose to either NaOH aqueous solutions or organic solvents (ethanol). Also, pH of the resulting suspensions kept under stirring are not reported. pH is a key parameter to consider when dealing with biomolecules, since they can easily undergo upon denaturation with consequent loss of their functionality. In order to fully preserve biocatalytic activity, immobilization strategies on Fe-BTC can be further improved. The contact between the enzyme and solvent can be minimized, or completely avoided, by using mechanochemical approach.

In our previous work,[70] LAG method was used to synthesize a Fe-BTC material, which showed better textural properties and crystallinity compared to the commercial product. Synthesis was performed grinding for one hour trimesic acid and ferric nitrate nonahydrate together with a small amount of an aqueous solution containing tetramethyl ammonium hydroxide pentahydrate ($TMAOH \cdot 5H_2O$). Despite the use of $TMAOH \cdot 5H_2O$, which act as a modulating agent avoiding recrystallization of trimesic acid, the pH remained acidic ($pH \approx 1$) throughout the synthesis, similarly to the solvo/hydrothermal protocol. Improvements still need to be addressed to exploit the unexpressed potential of mechanochemistry for biomedical applications. Hence, we questioned whether MOFs synthesis conditions could be efficiently *ad hoc* tailored depending on their final application.

We reported here the case study of Fe-BTC mechanochemically prepared for the first time under solvent-free, biocompatible conditions suitable for one-pot immobilization strategies. Avoiding the use of solvents, such synthesis procedure assesses both green chemistry criteria and bio-friendly targets, overcoming the limits given by acidic pH conditions and solvent-based methods. Mild pH range working conditions are attained without using any additional solvents but adding $TMAOH \cdot 5H_2O$ as solid rather than aqueous solution. Besides the lower costs and the eco-benefits of a solventless approach, the NG method enables high concentrations of $TMAOH \cdot 5H_2O$ without being limited by its solubility. Hence, milder pH conditions can be easily reached by adding a larger amount of base as solid, instead of requiring the use of more concentrated solutions and/or the addition of a higher volume of liquid, which would eventually turn the mechanochemical approach into a batch-based one. Despite being a solvent-free procedure, the presence of hydrated reactants releases liquid water during the reaction, which has been demonstrated to accelerate the mechanochemical conversion of reactants into products.[71] This is why, in contrast to anhydrous bases, such as NaOH pellets, TMAOH·5H_2O has to be preferred. Synthesis was performed in just 1 hour, at room temperature, at pH 3, 4 and 5, using different amounts of TMAOH·5H_2O as base and modulating agent.[72] Traditional harsh acidic conditions were overcome, avoiding any potential contact between solvents and biomolecules, opening up novel perspectives for the preparation of hybrid biocomposites in a single-step process.

2. Experimental

2.1 Materials

Iron(III) nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$, 98%), 1,3,5-benzenetricarboxylic acid (H_3BTC, 95%) and tetramethyl ammonium hydroxide pentahydrate (TMAOH·5H_2O, 97%) were supplied by Sigma Aldrich and used as received without further purification. Milli-Q deionized water with specific resistance (18.2 MΩ·cm) was obtained from a Millipore Milli-Q Ultrapure Water Purification System.

2.2 Methods

0.47 g of H_3BTC, 1.29 g of $Fe(NO_3)_3 \cdot 9H_2O$ and proper amounts of TMAOH·5H_2O (1.63, 1.81 or 1.99 g to obtain a pH of 3, 4 or 5, respectively) were place in a 32 mL Teflon coated grinding jar made of stainless-steel with 13.7 g of zirconia balls (10 mm diameter). No additional solvents were added. The reaction mixture was grinded for 1 hour using a Spex 8000 Mixer/Mill. The resulting dense orange slurry was dispersed in 20 mL of Milli-Q deionized water and the pH of the obtained dispersion was measured. The dispersion was centrifugated (2500 rpm; 10 minutes), the precipitate was washed two times with Milli-Q deionized water, and finally dried at room temperature for 48 hours under air. The resulting dry sample was orange-colored. To distinguish among the different working pH conditions, samples were labelled Fe-BTC_pH3, Fe-BTC_pH4 and Fe-BTC_pH5.

2.3 Characterizations

All samples were characterized by X-ray powder diffraction (XRPD), attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR), scanning electron microscopy (SEM), thermal analysis and nitrogen physisorption at -196°C. XRPD patterns were recorded on a Bruker D8 Advance Diffractometer using Cu-Kα radiation (V = 40 kV, I = 30 mA, λ = 1.54056 Å) in a scanning range between 4° and 80° 2-theta with a step size of 0.05° 2-theta. In order to obtain a satisfactory signal-to-noise ratio in the XRPD pattern an appropriate acquisition time was selected. ATR-FTIR spectra were collected on a Bruker Tensor 27 spectrophotometer within the 400-4000 cm^{-1} scanning region. SEM images were obtained using a FEI Quanta 200 microscope. Thermogravimetric analysis (TGA) and differential scanning calorimetry analysis (DSC) were performed on a Perkin-Elmer STA 6000 simultaneous thermal analyzer in a temperature range between 25 and 850 °C (10 °C min^{-1}) under oxygen flow (40 mL min^{-1}). Nitrogen physisorption was carried out in a Sorptomatic 1990 CE apparatus (Fisons Instruments) at -196 °C. Before the measurement, all the samples were outgassed at 150 °C under vacuum for 17 hours. Specific Surface Area (SSA) was estimated from adsorption data by using Dubinin-Radushkevich (DR) method. Pore size distribution was determined by applying Horvath-Kavazoe (HK) equation to the adsorption branches of the nitrogen isotherms.

3. Results

The effect of pH on the microstructure, thermal stability and textural properties of the material was investigated. Figure 1 shows the XRPD patterns of the samples Fe-BTC_pH3, Fe-

BTC_pH4 and Fe-BTC_pH5 synthesized at different pH values. All samples present a similar diffraction pattern in terms of position of diffraction peaks, in agreement with XRPD patterns elsewhere reported for Fe-BTC frameworks.[73,74] An intense diffraction peak centered at 10.65° 2-theta with a visible shoulder at around 14.30° 2-theta was observed for all samples. The following peaks at ca. 18.76, 23.83, 28.09, 33.36, 42.49° 2-theta are broader and less intense than the first one. The presence of broad diffraction peaks is consistent with the disordered structure of Fe-BTC material compared to crystalline MIL-100(Fe),[75] which has been ascribed to the presence of terminal carboxyl groups belonging to partially deprotonated or extra-framework trimesic acid moieties, interrupting the order of the network.[58] The existence of these COOH groups, which prevent the formation of a crystalline structure, is confirmed by the presence of a band at 1703 cm^{-1} (w) in the ATR-FTIR spectra of Fe-BTC_pH3, Fe-BTC_pH4 and Fe-BTC_pH5 (Figure 2). It is interesting to notice that the relative intensity of the carbonyl band gradually decreases with increasing pH, which agrees with the presence of larger amounts of deprotonated form of trimesic acid at higher pH values. Despite this slight difference, the spectra of the samples synthetized at different pH values are similar to each other as well as to the results elsewhere reported for iron(III) trimesate MOFs[76]. Table 1 reports ATR-FTIR experimental bands (cm^{-1}) observed for Fe-BTC_pH3, Fe-BTC_pH4 and Fe-BTC_pH5 in the 4000-400 cm^{-1} region. The broad band in region 3600-3100 cm^{-1} is ascribable to the stretching vibrations of adsorbed water molecules and hydroxyl groups and water molecules coordinated to iron octahedra.[77,78] The relative intensity of this band slightly decreases at higher pH values, suggesting the presence of larger amounts of adsorbed water in Fe-BTC_pH3, compared to Fe-BTC_pH4 and Fe-BTC_pH5. Nevertheless, this is a negligible difference between samples since water adsorption is strongly dependent on the extent of humidity and exposure time to air of the samples. The weak band at 3080 cm^{-1} is due to the aromatic C–H stretching vibrations. The band at 1625 cm^{-1} is attributed to the C=O stretching of carboxylate groups, whereas the bands at ca. 1564 and 1371 cm^{-1} are respectively assigned to the asymmetric and symmetric stretching of the O–C–O group. The bands at 759 and 706 cm^{-1} are ascribable to the C–H bending vibrations of aromatic rings, while at 463 cm^{-1} we observe the stretching of Fe-O bonds.[79–81] Despite the different pH conditions during the synthesis, all samples show no relevant differences when comparing microstructure by XRPD and ATR-FTIR. The thermal stability of the samples was also investigated. The TG curves under oxygen flow, the corresponding derivative curves (dTG) and the DSC curves for Fe-BTC_pH3, Fe-BTC_pH4 and Fe-BTC_pH5 are reported in Figure 3. TG curves of samples (Figure 3a) show three weight losses between 25 and 850°C, as reported elsewhere.[82,83] The first weight loss occurs up to 150°C and is attributed to the loss of adsorbed water molecules. This first loss is higher for Fe-BTC_pH3 compared to Fe-BTC_pH4 and Fe-BTC_pH5, further confirming the variable amounts of adsorbed water observed by ATR-FTIR spectroscopy. The second weight loss occurs over the range 150 and 260 °C and is related to the departure of water molecules coordinated to iron trimers. The third weight loss between 260 and 450°C arises from the collapse of the framework into hematite. The decomposition by combustion of the framework is demonstrated by the presence of an intense exothermic peak at around 350°C in the DSC curves of the samples (Figure 3c). Nitrogen adsorption/desorption isotherms and pore size distributions for Fe-BTC_pH3, Fe-BTC_pH4 and Fe-BTC_pH5 are reported in Figure 4. All samples present a I-type isotherm, which is typical of microporous material.[84] Fe-BTC_pH3 and Fe-BTC_pH4 display a plateau at ca. $p/p^0 = 0.12$, while the plateau is reached at lower relative pressure ($p/p^0 = 0.01$) and at lower adsorbed volume for Fe-BTC_pH5. Further distinction can be established for the isotherm of Fe-BTC_pH4, which differs from the other samples for the presence of a hysteresis loop at p/p^0 0.83-0.99, that we hypothesize due to capillary condensation that occurs because of interparticle mesopores. Fe-BTC_pH4 shows a relatively higher mesopore and macropore volume than Fe-BTC_pH3 Fe-BTC_pH5 (Table 2), indicative of a different degree of particle aggregation for Fe-BTC_pH4, which gives rise to interparticle meso- and macropores, as also confirmed by SEM analysis. As shown in Figure 5, all samples are found to be formed by agglomeration of particles covered by much smaller particles. However, the presence of such smaller particles is particularly pronounced for Fe-BTC_pH4, giving rise to some interparticle mesoporosity, in agreement with the existence of a hysteresis loop in the mesoporous region of the isotherm. The optimal results in terms of specific surface area (SSA) (742 m^2/g) and micropore volume (0.260 cm^3/g) were reported for Fe-BTC_pH3. We observed a decrease of both SSA and micropore volume as the pH increased (Table 2). The decrease in SSA and the micropore volume of the material with increasing pH can be explained using the framework formation proposed by Seo and co-workers.[85] The first step is comprised of the hydrolysis of iron(III) ions and trimesic acid, followed by condensation. The hydrolysis of Fe(III) ions produces $[Fe(H_2O)_{6-n}(OH)_n]^{(3-n)+}$ octahedra, which further arrange to form trinuclear metal units. Once trimeric clusters are formed, carboxylic moieties of trimesic acid can orient to complexed water molecules using H-bonding interactions. Full deprotonation of trimesic acid ($pK_{a1} = 3.12$; $pK_{a2} = 3.89$; $pK_{a3} = 4.70$)[85] is needed to promote condensation, leading to the formation of μ_3-O corner sharing iron octahedra assembled to constitute the supertetrahedral building blocks of MIL-100(Fe). Even though trimesic acid deprotonation and, thus, condensation is favored at basic conditions, low pH allows a better control of the reaction kinetics through the lower extent of hydrolysis of carboxyl moieties.[86] In fact, a slow rate of condensation leads to a more ordered arrangement in space of carboxylic moieties which can orient properly towards complexed water molecules by H-bonding interactions, leading to the formation of highly porous and ordered solids. Furthermore, trimeric iron(III) clusters, built in the very early stage of the reaction, are stabilized over monomeric octahedra in acidic conditions. This mechanism clarifies why we attained the best results at pH 3 and why acidic conditions, high pressure and long reaction time are required for the traditional synthesis of well-defined and crystalline MIL-100(Fe). Therefore, as the pH increases, despite having a lower extent of extra-framework carboxylic groups which could be assumed as a hint of a more ordered structure, the rate of condensation increases, preventing the effective formation of a considerable permanent porosity of the sample. This explains the decreasing SSA and micropore volume observed at increasing pH from 3 to 5.

4. Discussion

The modulation of synthesis conditions of a support material while targeting *in situ* immobilization strategies can be very challenging. This is the case for pH-sensitive guest molecules (e.g. drugs, nucleic acids, proteins), which can decompose under harsh conditions. Moreover, finding the balance between chemical-physical integrity of the support and activity retention of biomolecules could be very discouraging, especially when conventional solution-based methods under harsh conditions are already consolidated for the preparation of the support. Solvent-free approaches represent an outstanding alternative to solution-based procedures to achieve *in situ* encapsulation. Here, we demonstrated how efficiently the traditionally harsh synthesized Fe-BTC MOF can be prepared in the solid state under mild, green and bio-friendly conditions. Avoiding the use of solvents, this approach is particularly feasible for one-pot immobilization of biomolecules. Compared to solution-based methods, which typically require the use of water[66] and/or organic solvents (ethanol)[67], any bulk influence on the guest molecules, which could lead to denaturation and activity loss, is further prevented. Microstructure and thermal stability are not affected by the different working pH conditions and are closely consistent with reported results of Fe-BTC MOFs synthesized by conventional methods.[50,68] Textural properties of the material have shown to be easily tunable by varying the amount of base. Hence, the amount of base used during the synthesis can be conveniently adapted case by case depending on the balance between the optimal conditions to retain the stability and functionality of a certain biomolecule and the resulting porosity of the MOF support. Specific surface areas are highly indicative of the biomolecule loading entity. However, N_2 physisorption analysis and corresponding SSA values are reported for a few enzyme–Fe-BTC composites obtained via solution-based methods.[65,67,69] SSA observed for Fe-BTC_pH3 (742 m^2/g) is comparable with those reported for Fe-BTC by Gascon et al. (740 m^2/g,[65] 753 m^2/g)[66], and much higher compared to those observed by Zhao et al. (51 m^2/g). Given similar physical-chemical characteristics under same temperature and pressure conditions (RT, ambient pressure) and comparable reaction times (1 h in the present work vs 10 min[65], 30 min[67] or 4 h[50]), a solventless pathway should be preferred. Fe-BTC materials are particularly attractive in food industry, environment safety and biomedicine since Fe is a biocompatible and safe metal ion. Secondly, such iron(III) trimesate MOFs possess enzymatic-mimic activity, avoiding the need to immobilize multiple enzymes in case of application for cascade reactions. Despite such remarkable advantages, only a few studies reported the use of Fe-BTC based enzyme-MOF composites obtained via *in situ* immobilization for the above-mentioned applications[49,50,67]. Moreover, attempts to integrate biomacromolecules in general within MOFs have been limited only to solvent resistant biomacromolecules.[87] The solvent-free method we propose can help further emerging the unexpressed potential of iron(III) trimesate MOFs as immobilization supports, especially on the industrial level. Indeed, a solventless pathway is appealing to industrial scale-up, reducing the environmental footprint of the process while ensuring the same efficiency of conventional pathways. This innovative solvent-free synthesis protocol demonstrated that the use of any solvents is completely unnecessary, representing an unprecedented breaking point compared to conventional batch-based methods. It can be adapted to the *in situ* encapsulation of a variety of guest molecules. The absence of solvents, which could penetrate into the active sites changing the local environment of biomolecules, together with the possibility to *ad hoc* modulate the mildness of the reaction mixture according to the stability of the guest and the desirable porosity of the support, can afford an outstanding protection from biological, and chemical degradation with maintenance of bioactivity. Such method looks towards a full retainment of biomolecules functionality while protectively coating them by porous and robust supports. This represents a benchmark for *ex vivo* applications of biomolecules, whose use is often limited by an intrinsic loss of activity due to the immobilization process. Indeed, biomolecules stability is affected by physical, biological, and chemical factors, requiring careful evaluation of the immobilization conditions (pH, temperature, concentrations). In this regard, the encapsulation of pH-sensitive molecules will be achievable in one-step by simply adding the guest together with the support's precursors in the solid state. Therefore, not only the preparation of novel biocomposites would be remarkably easier and cheaper compared to batch-based methods, but it would also carefully assure the maintenance of both guest and host integrity and functionality. On the other hand, such simple pH-dependent fine tuning of MOF porosity will also allow to modulate the accessibility and diffusion of the substrates to the active sites by changing synthetic parameters. This is crucial to develop highly performing biosensors, biocatalysts and drug delivery systems with enhanced activity and selectivity. Moreover, this approach can aim the *in situ* encapsulation of biomolecules which has been hindered by the immobilization conditions (harsh pH, solvent incompatibility, long reaction times, etc). For instance, antibiotics including doxycycline and tetracycline,[88] nucleoside reverse transcriptase inhibitor drugs against the human immunodeficiency virus (HIV), such as azidothymidine and lamivudine,[89] anti-cancer drugs (e.g. docetaxel),[90] 3-azido-D-alanine (d-AzAla) for the metabolic labeling of the Methicillin-resistant bacteria *Staphylococcus aureus*,[91] therapeutic RNA for the treatment of various diseases like cancer or neurological disorders,[92] have been immobilized on iron(III) trimesate MOFs exclusively by post-synthetical surface adsorption. Providing mild bio-friendly conditions, our protocol would address the *in situ* immobilization of such drugs in a single-step process. Post-synthesis steps would be avoided, and the drugs resulted encapsulated within the pores of the support. Such one-pot pore encapsulation help dosing drug release rather than leading to a burst effect given by the rapid release of molecules weakly attached on the surface, improving the overall drug bioavailability. Hence, this study looks at a new generation of biocomposites to produce pioneering biosensors, biocatalysts and drug delivery systems with minimum functionality loss compared to free biomolecules. Moreover, it indicates how efficiently the synthesis of a Fe-BTC materials can be tailored depending on their final application. Such approach can be potentially adapted to an infinite variety of MOFs and guest molecules, making the fabrication of biomolecule-MOF-based composites a facile, green, and usual procedure, and we hope to inspire more research and development in this direction.

5. Conclusions

We successfully demonstrated the tailorability of iron(III) trimesate MOF synthesis to solvent-free, biocompatible and sustainable reaction conditions for biomedical applications. An innovative bio-friendly mechanochemical route without adding solvents, over a mild pH range and with just 1 hour reaction was proposed. The effect of pH was evaluated on the basis of the formation mechanism of this material, pointing-out that the porosity of the material can be tuned by simply varying the amount of base. The use of solvents has proven to be superfluous for the obtainment of the framework, paving the way for numerous novel applications of Fe-BTC materials and biomolecule-MOF composites in biosensing, biocatalysis and drug delivery. This is of main interest, especially in recent years, in which finding alternative and greener options urges to be addressed in every field of human life. Moving towards new strategies that involve less steps and reduce, or completely avoid, the use of solvents, is demanding but still essential for sustainability. Further research is currently in progress to perform one-pot synthesis with *in situ* immobilization of enzymes on Fe-BTC to prepare hybrid composite materials for biomedical purposes.

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Declaration of interest

We have no conflicts of interest to disclose.

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Table 1. ATR-FTIR experimental frequencies (cm^{-1}) for Fe-BTC_pH3, Fe-BTC_pH4 and Fe-BTC_pH5 samples.

Exp. Freq. Fe-BTC_pH3 (cm^{-1})	Exp. Freq. Fe-BTC_pH4 (cm^{-1})	Exp. Freq. Fe-BTC_pH5 (cm^{-1})
3370 (m)	3390 (w)	3380 (vw)
3080 (vw)	3080 (vw)	3080 (vw)
1710 (w)	1700 (w)	1710 (w)
1620 (s)	1620 (s)	1610 (s)
1570 (ms)	1560 (ms)	1560 (ms)
1440 (s)	1440 (s)	1440 (s)
1370 (vs)	1370 (vs)	1370 (vs)
1100 (w)	1100 (w)	1100 (w)

940 (w)	940 (w)	940 (w)
760 (m)	760 (m)	760 (m)
710 (s)	710 (s)	710 (s)
620 (w)	620 (w)	620 (w)
460 (s)	460 (s)	460 (s)

Table 2. Specific Surface Area (SSA) and macro-, meso- and micropore volume of Fe-BTC_pH3, Fe-BTC_pH4 and Fe-BTC_pH5.

Sample	SSA[a] (m^2/g)	Micropore volume[b] (cm^3/g)	Mesopore volume[b] (cm^3/g)	Macropore volume[b] (cm^3/g)
Fe-BTC_pH3	742	0.260	0.007	0.003
Fe-BTC_pH4	450	0.162	0.089	0.048
Fe-BTC_pH5	57	0.020	0.008	0.002

[a] Calculated by DR method. [b] Calculated through HK method.

Figure 1. XRPD patterns of Fe-BTC_pH3, Fe-BTC_pH4 and Fe-BTC_pH5 recorded from 5 to 80° 2-theta using a CuKα X-ray radiation.

Figure 2. ATR-FTIR spectra of Fe-BTC_pH3, Fe-BTC_pH4 and Fe-BTC_pH5 from 400 to 4000 cm^{-1}.

Figure 3. TG (a), dTG (b) and DSC (c) curves and of Fe-BTC_pH3, Fe-BTC_pH4 and Fe-BTC_pH5 samples, obtained in the temperature range between 25 and 850°C (10°C min^{-1}) under oxygen flow (40 ml min^{-1}).

Figure 4. Nitrogen adsorption/desorption isotherms obtained at -196°C and pore size distributions (inset) of Fe-BTC_pH3, Fe-BTC_pH4 and Fe-BTC_pH5.

Figure 5. SEM images of Fe-BTC_pH3 (a), Fe-BTC_pH4 (b) and Fe-BTC_pH5 (c).