MIL-53 (Al) metal-organic frameworks as potential drug carriers

O Yu Griaznova¹, ², *https://orcid.org/0000-0002-6180-7444, I V Zelepukin¹, ², https://orcid.org/0000-0003-0209-2116, G V Tikhonowski³, https://orcid.org/0000-0003-4998-1631, V N Kolokolnikov³, https://orcid.org/0000-0003-0559-9916, S M Deyev¹, ², https://orcid.org/0000-0002-3952-0631

¹ National Research Nuclear University “MEPhI”, Moscow, Russia
² Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of Russian Academy of Sciences, Moscow, Russia
³ Federal State Budgetary Scientific Institution “Federal Scientific Agroengineering Center VIM”, Moscow, Russia
* Corresponding author. E-mail: olgayugryaznova@yandex.ru

Abstract. One of the challenges of the medicine is to improve the chemical stability of drugs and to prevent their premature biodegradation before reaching the therapeutic target. Various nanoparticles were used to solve this problem, but low drug loading efficiency limited their biomedical applications. Metal organic frameworks are promising candidates for drug delivery since they have extremely high surface area and regular porosity. In this study, we prepared high-crystalline MIL-53 frameworks based on aluminium and 2-aminoterephthalic acid by microwave-assisted synthesis and evaluated their properties as drug carriers. Drug loading of chemotherapeutic and diagnostic molecules of different nature riches value of 34% by particle weight, significantly higher than those of other reported solid nanoparticles. Therefore, our results make MIL-53 (Al) frameworks promising candidate for drug delivery.

1. Introduction
One of the challenges that biomedicine is facing now are the toxicity effects to healthy tissues caused by systemic administration of therapeutic drugs. One way to solve this problem is to encapsulate therapeutic molecules to a vehicle, that will specifically accumulate in the target organ [1,2]. Additionally, this will prevent therapeutic molecules from early biodegradation, thereby increasing the stability of the drug. Most metal nanoparticles (NPs) used in drug delivery have low loading efficiency – usually less than 5% by particle weight [3]. Hence, it is necessary to design new drug delivery systems, which will have higher drug capacity.

Metal-organic frameworks (MOFs) are a new class of highly porous hybrid organic-inorganic materials, which are self-assembled from secondary building units (SBUs) – multivalent metal cations or their clusters and organic ligands. Due to their extremely high specific surface area exceeded 6000 m²/g [4] and regular porosity they were extensively used for gas sorption, separation of gases, catalysis, waste removal, gene delivery, etc. [5,6] The key future of MOFs for all their applications is their adsorption performance. Therefore, MOFs are the great alternative to metal NPs in their use as drug delivery agents.

One of the barriers in the MOF application in vivo is their potential toxicity. Long-term nanoparticle toxicity to the organism partially comprises from the toxic effects of its constituent elements [7], SBUs in case of MOFs. Many MOFs are built from heavy metals, such as Cd²⁺, Tb³⁺, Eu³⁺, etc., [8,9] which...
are highly toxic [10,11]. But this problem can be easily solved, as certain topologies of MOFs can be formed by different metal cations. For example, metal-organic frameworks MIL-53 can be synthesized from different trivalent metal ions: Cr, Al, Fe. MIL-53 frameworks have high loading efficiency. For instance, MIL-53 frameworks based on Cr$^{3+}$ ions were used as Ibuprofen carriers with drug loading efficiency ~20% by particle weight [12]. Despite of MIL-53 (Cr) can be loaded with therapeutic agents, it is not suitable for drug delivery, since chromium is highly toxic, with LD$_{50}$ 140-422 mg Cr$^{3+}$/kg of body weight [13,14].

In this study we prepared amino functionalized MIL-53 frameworks based on Al$^{3+}$ ions. This metal has smaller toxicity, i.e. LD$_{50}$ for aluminum salts is in the range 3.3-3.6 g/kg of body weight [15], which make them better choice for biomedical applications.

Drug loading efficiency of MIL-53 (Al) for different molecules was investigated. These MIL-53 (Al) frameworks show high loading efficiency for various small molecules: fluorescent dyes, photosensitizers, and vitamins, up to 35% by particle weight. Thus, our results make MIL-53 (Al) metal-organic frameworks perspective container for drug delivery applications.

2. Materials and Methods

2.1. Materials.
All materials were purchased from Sigma-Aldrich (USA): 2-Aminoterephthalic acid, Aluminium chloride hexahydrate, dimethylformamide (DMF), Sulforhodamine B monosodium salt, Rhodamine b, Trypan blue, Rhodamine 6G, Bromophenol blue, Acridine Orange hydrochloride hydrate, Indocyanine green, Fluorescein, Vitamin B$_{12}$, Riboflavin 5’-monophosphate sodium salt hydrate.

2.2. Methods.

2.2.1. Synthesis. The synthesis of MIL-53 (Al) was adapted from [16]. Aluminium chloride hexahydrate (0.51g) was dissolved in 40 ml DMF and stirred for 30 min, until the precipitate was completely dissolved. Then 2-aminoterephthalic acid (0.56 g) was added to the solution. Mixture was heated at 130 °C for 20 min by microwave irradiation using Monowave 400 reactor (Anton Paar GmbH, Austria). The resulting particles were washed via centrifugation with hot methanol and hot DMF 3 times each to remove unreacted chemicals.

2.2.2. Hydrodynamic size and ζ-potential analysis. Measurements were performed in distilled water for size and in 10 mM NaCl for ζ-potential analysis using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK) device. Distribution by numbers was used to show hydrodynamic size of the particles.

2.2.3. X-ray diffraction. The crystal structure of MOFs was analysed by the powder X-ray diffraction (PXRD) technique, using Cu-K$_a$ radiation LabX XRD-6000 X-ray diffractometer (Shimadzu, Japan), operated with 30 mA current and 40 kV voltage.

2.2.4. UV-Vis spectroscopy. Absorbance spectra in 350-800 nm diapason were measured using an Infinite M1000Pro microplate reader (Tecan, Switzerland).

2.2.5. Scanning electron microscopy. The MAIA3 (Tescan, Czech Republic) electron microscope was used to obtain micrographs of the particles. Particles in distilled water were placed on the silicon grid and dried under the air. Measurements were performed at accelerating voltage of 20.0 kV using In-Beam secondary electron mode.

2.2.6. Drug loading measurements. To load various molecules and study the drug loading of nanoparticles, 10 mg of MIL-101 (Fe) were incubated with 10 mg of the substance dissolved in 1 ml of deionized water for 1 h at room temperature. Then the solution was centrifuged at 12000 g for 6 min and the supernatant was collected for further study of the absorption spectrum. Drug concentration was
quantified using calibration curves. To calculate drug loading efficiency, mass of the adsorbed drug was normalized by the particle mass.

3. Results and discussion

3.1. Synthesis and characterization.

The MIL-53 (Al) frameworks were synthesized by microwave assisted synthesis as described in materials and methods section. There are several approaches in heating of solution in solvothermal synthesis: conventional heating in the oven, sonication- and microwave-assisted synthesis. Microwave-assisted synthesis allows to obtain MOFs much faster than conventional solvothermal approach. Reported synthesis of MIL-53 (Al) in the oven takes 48 hours [17], while microwave synthesis used in this study takes only 30 min.

Scanning electron microscopy allows to see the surface morphology of particles, such as shape and size. The SEM micrographs of MIL-53 (Al) frameworks are presented in Fig.1a-c. The obtained MIL-53 (Al) particles have spike-like surface and irregular shape.

The distribution of particles’ hydrodynamic size by number is presented in Fig.1d. Mean hydrodynamic size of MIL-53 (Al) particles is equal to (870 ± 110) nm.

Colloidal stability of particles in the water solution can be estimated by magnitude of its ζ-potential. Particles with high values of ζ-potential by magnitude (over 30 mV) either positive or negative are thought to be stable [18]. In our study MIL-53 (Al) frameworks have good colloidal stability, as its ζ-potential is equal to (35 ± 5) mV, the distribution is shown in Fig.1e.

![Figure 1. Characterization of metal-organic frameworks MIL-53 (Al): a-c) SEM micrographs, scale bar – 500 nm; d) hydrodynamic size distribution by number; e) ζ-potential; f) powder X-ray diffraction spectra.](image)

Crystal structure of particles was examined by powder X-ray diffraction analysis. PXRD pattern of microwave synthesized particles MIL-53 (Al) is shown in Fig.1f. Resulting pattern of MOFs has characteristic peaks on 8.8, 11.7, 14.9 and 17.6 2θ degrees as reported elsewhere [19,20], which confirms that obtained particles have crystal structure of MIL-53.
3.2. Drug loading.

To investigate drug loading of MIL-53 (Al) particles for different molecules they were incubated with water solution of dyes for 1 h at room temperature without stirring. After centrifugation the supernatant was collected. Then using Beer–Lambert law the concentration of dye in supernatant was calculated by absorbance coefficient at wavelength corresponding to maximum absorbance. The table 1 shows drug loading efficiency of MIL-53 (Al) frameworks for different molecules and application of these molecules. Drug loading efficiency was calculated as the ratio of dye adsorbed mass to the MIL-53 (Al) mass. Different dyes, photosensitizers, and vitamins were chosen as model molecules to analyze drug loading efficiency of MOFs.

Different rhodamine dyes can be used for fluorescent analysis, imaging, and in different sensing systems [21]. Bromophenol blue, Acridine orange, Indocyanine green and Fluorescein can be used as dyes, but also as photosensitizers (PSs) for photodynamic therapy [22–25]. These molecules can produce reactive oxygen species (ROS) in the presence of oxygen molecules. ROS are highly cytotoxic and can be used for cancer and bacteria sepsis treatments. It is possible to increase accumulation of PSs in specific tissues and organs using containers to deliver them, rather than using systematic administration.

**Table 1.** Drug loading efficiency of various molecules into the MIL-53 (Al) particles

| Molecule                  | Drug loading (w/w, %) | Type             |
|---------------------------|-----------------------|------------------|
| Sulforhodamine b          | (18.65 ± 0.02)        | Dye              |
| Rhodamine b               | (19.21 ± 0.02)        |                  |
| Trypan blue               | (16.37 ± 0.05)        |                  |
| Rhodamine 6G              | (4.25 ± 0.01)         |                  |
| Bromophenol blue          | (22.387 ± 0.001)      | Dye, photosensitizer |
| Acridine orange           | (14.01 ± 0.03)        |                  |
| Indocyanine green         | (25.6 ± 0.9)          |                  |
| Fluorescein               | (8.04 ± 0.02)         |                  |
| Vitamin B<sub>12</sub>    | (34.37 ± 0.04)        | Vitamin          |
| Riboflavin mononucleotide | (35.54 ± 0.01)        |                  |

MOFs MIL-53 (Al) has high drug loading for both Vitamin B<sub>12</sub> and Riboflavin mononucleotide about 35% by particle weight. Drug loading of MIL-53 (Al) for such dyes as Sulforhodamine b, Rhodamine b is approximately equal to 19% and Trypan blue is 17%, for Rhodamine 6G – 4% w/w.

Comparing the drug loading of different photosensitizers, it can be seen that MOFs have the highest loading efficiency for Indocyanine green (26% w/w). Particles of MIL-53 has drug loading for Bromophenol blue, Acridine orange and Fluoresceine - 22%, 14% and 8%, respectively. MIL-53 (Al) frameworks exhibit higher loading efficiency of Acridine orange than record value reported previously—about 13% on MnO<sub>2</sub> nanoparticles [26]. Despite the fact that MIL-53 (Al) metal-organic frameworks have the lowest adsorption capacity of Fluorescein among other molecules in this group, less than 10%, these MOFs have exceptional drug loading efficiency comparing to other carriers. For example, polymeric hybrid silica particles can adsorb only 0.2% w/w of Fluorescein [27].
4. Conclusion
In this paper, aluminium based metal organic frameworks were synthesized by microwave assisted solvothermal method. The particles have a crystal structure corresponding to the MIL-53 crystal type. These particles can be loaded with various molecules: dyes, photosensitizers, and vitamins with high 4-36% drug loading efficiency, which makes them promising candidates for use as containers for drug delivery. Also, MIL-53 frameworks can be used for removal of pharmaceutical waste from water, as they can adsorb molecules from water solution.

5. Acknowledgements
The work was supported by Russian Science Foundation grant no. 19-72-30012 (nanoparticles synthesis, characterization, and drug loading efficiency) and by the Ministry of Science and Higher Education of the Russian Federation, agreement no. 075-15-2020-774 (XRD study).

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