Synthesis of bismuth-based coordination polymer for biomedical applications

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Abstract. Metal organic frameworks (MOFs) are of great interest for biomedicine due to their high loading capacity of various drugs, dyes, and other small molecules. In vivo application of MOFs requires small sizes of nanoparticles and their high colloidal stability. Here we designed the first nano-sized MOFs composed of bismuth and trimesic acid by a rapid microwave-assisted solvothermal method. After coating of the prepared nanoparticles with polyacrylic acid they showed improved colloidal stability in aqueous solutions. These particles have 2.2 times higher X-ray attenuation ability than a clinically used BaSO4 agent. Also, they demonstrate high loading efficiency for organic dyes: 35.5 % w/w for Rose bengal and 17.9 % w/w for Rhodamine B.

1. Introduction
Bismuth is one of the safest heavy elements, which complexes are widely used in biomedicine. The majority of bismuth compounds possess low toxicity due to their weak solubility in biological fluids [1]. Moreover, they show the ability to inhibit the growth of several pathogenic bacteria, e.g. Helicobacter pylori [2]. Hence, bismuth complexes were actively studied for treatment of gastrointestinal diseases, bacterial and fungal infections.

Also, bismuth compounds are considered as potential contrast agents for computed tomography (CT) imaging [3]. Various adverse effects of clinically used iodine- and barium-based CT contrast agents have been reported so far [4], which limits their frequent usage. However, due to a stronger X-ray absorption ability of bismuth at the broad range of medically relevant X-ray tube settings [5] and its biological safety, an appropriate contrast may be obtained at lower doses of bismuth-containing agents.

In the recent decade, a wide variety of bismuth coordination complexes have been designed due to the flexible coordination geometry of bismuth and large radius of Bi\textsuperscript{3+} ions [6]. One of the most promising classes of bismuth based materials for biomedicine are metal-organic frameworks (MOFs), which are structured porous compounds with metal nodes coordinated to organic ligands to form multidimensional structures with large surface area [7].

MOFs based on bismuth and trimesic acid (Bi-BTC) have been extensively studied and a large amount of diverse crystal structures were obtained by changing solvents and conditions of a microwave
heating [8]. Some Bi-BTC coordination polymers were synthesized solvothermally [9], however, microwave-assisted routes demonstrated crystallization of the desired product far more quickly.

In general, for efficient use of metal-organic particles in biomedicine their size should be decreased to nanoscale, because particles of larger sizes are subject to the rapid clearance by the immune system [10,11]. However, most of the Bi-BTC coordination compounds, according to the published reports, have particle size of a few microns or higher and plate-like or rod-like shape, which make them hardly applicable for biomedical purposes due to increased probability of cell and tissue damage [12]. Thus, an approach for nanosizing and changing morphology of particles should be proposed in order to design structures suitable for \textit{in vivo} applications.

In this study, we synthesized nano-sized Bi-BTC frameworks, which retain colloidal stability in water. These MOFs show 2.2-fold better X-ray attenuation than clinically used barium sulfate and have extremely high loading efficiency for Rhodamine B and Rose bengal dyes.

2. Experiment

2.1. Materials
Bismuth (III) nitrate pentahydrate ($\text{Bi(NO}_3\text{)}_3\cdot 5\text{H}_2\text{O}$, $\geq 98.0\%$), trimesic acid ($\text{H}_3\text{BTC}$, $\text{C}_9\text{H}_6\text{O}_6$, $95\%$), hexadecyltrimethylammonium bromide (CTAB, $\text{CH}_3(\text{CH}_2)_15\text{N(Br)(CH}_3)_3$, $\geq98\%$), N,N-dimethylformamide (DMF, $\text{C}_3\text{H}_7\text{NO}$, $\geq99\%$), methanol ($\text{CH}_3\text{OH}$), polyacrylic acid (PAA, average Mw $\approx 5100$), barium sulfate ($\text{BaSO}_4$, $99\%$), Rose bengal ($\text{C}_{20}\text{H}_{22}\text{Cl}_4\text{I}_4\text{Na}_2\text{O}_5$, $95\%$), Rhodamine B ($\text{C}_{28}\text{H}_{31}\text{ClN}_2\text{O}_3$, $>96\%$) were purchased from Sigma-Aldrich, USA.

2.2. Synthesis of Bi-BTC MOFs
77.6 mg of $\text{Bi(NO}_3\text{)}_3\cdot 5\text{H}_2\text{O}$ (0.16 mmol), 21.1 mg of $\text{H}_3\text{BTC}$ (0.1 mmol) and 3 mg of CTAB were mixed with 3 ml of methanol in a 6 ml glass vial. The mixture was shaken for 5 min and then 1 ml of DMF was added. The vial was shaken again until the mixture became colorless. The vial was placed in a microwave oven and heated at 120 °C for 5 min under stirring at 800 rpm. Then, white crystals were washed sequentially by DMF, MeOH and distilled water. For characterization of Bi-BTC the product was dried under vacuum overnight.

2.3. PAA functionalization of Bi-BTC
Bi-BTC powder was added to a PAA aqueous solution (50 mg/ml) to the 1 mg/ml particle concentration. The suspension was heated at 70 °C for 30 min. PAA-coated particles were collected by centrifugation (10 000 g, 5 min) and washed several times with distilled water.

2.4. Characterisation of Bi-BTC MOFs
Morphology and size of the synthesized material were characterized using scanning electron microscopy (SEM) on a Tescan MAIA3 (Tescan, Czech Republic) device at 30 kV accelerating voltage. The samples were dissolved in ethanol, placed onto a silicon wafer and then air-dried. The X-ray diffraction pattern (XRD) was collected on a RADIANT-DR2 diffractometer (ZAO “STC Expert Center”, Russia). The infrared spectroscopy (FTIR) was performed using FSM 2203 Fourier-transform infrared spectrometer (Infraspek, Russia) with an ATR unit. Hydrodynamic size and $\zeta$-potential of the particles in water was measured by dynamic and electrophoretic light scattering (DLS) techniques using a Malvern Zetasizer Nano ZS device (Malvern Instruments, UK).

2.5. CT attenuation measurements
The samples of 1% agarose solutions with different Bi-BTC and BaSO$_4$ concentrations were added to 1.5 ml Eppendorf tubes and placed in an imaging chamber of an IVIS Spectrum CT imaging system (PerkinElmer, USA). CT images were obtained at 50 kV tube voltage and 1 mA current. The data was reconstructed and the mean intensities of regions of interest were measured for each sample. These
values were finally converted into the Hounsfield Units (HU) to determine the enhancement in X-ray attenuation.

2.6. Dye encapsulation by Bi-BTC MOFs

The sample of Bi-BTC (1 mg) powder was dissolved in the aqueous solution of Rose bengal (5 mg/ml, 1 mg) or Rhodamine B (3.3 mg/ml, 1 mg). The suspension was incubated at room temperature under stirring for 3 h. Then, supernatant was separated from particles and its absorbance was measured at 560 nm for Rose bengal and 554 nm for Rhodamine B using an Infinite M1000 PRO spectrophotometer (Tecan, Austria). UV-Vis spectra of Bi-BTC before and after dye encapsulation were obtained after intensive washing of particles with water (3 times) and ethanol (2 times). The concentrations of the dyes in supernatant were calculated using calibration curves. Loading efficiency was determined by the following formula:

$$\text{LE} (%) = \frac{(C_0 - C) \times V}{m} \times 100$$

where $m$ is the mass of the powder, $C$ is the dye concentration after encapsulation, $C_0$ and $V$ are the initial concentration of the dye and volume of the solution, respectively.

3. Results and discussion

Bi-BTC crystals were obtained by the rapid and facile microwave-assisted synthesis in MeOH/DMF solvent mixture. CTAB molecules were added to the reaction before synthesis to stabilize particles. The SEM images demonstrated that the as-prepared particles were mostly spherical with an average diameter of $d = 455 \pm 95$ nm (figure 1 (a), (b)). We suppose that CTAB prevents excessive crystal growth and formation of large rod-like particles as it was reported for a similar solvothermal synthesis of Bi-BTC particles in DMF/MeOH mixture without a surfactant reported in [13].

The XRD pattern of the as-prepared Bi-BTC MOFs also was isomorphic to the one presented for Bi-BTC in [13], conserving the majority of the most intense diffraction peaks (figure 1 (c)). The strong intensity of peaks indicated the high crystallinity of the particles. The presence of BTC in the molecular structure of the sample was indicated by measuring the FTIR spectrum (figure 1 (d)). The sample had the strong bands of carboxylate groups at 1651 cm$^{-1}$ and 1436 cm$^{-1}$ which correspond to the asymmetric ($\nu_{\text{asym}}$) and symmetric ($\nu_{\text{sym}}$) O-C=O stretching, respectively. According to [14], H$_3$BTC has $\nu_{\text{asym}}$ at 1723 cm$^{-1}$ and $\nu_{\text{sym}}$ at 1450 cm$^{-1}$ with the broad bands in the range of 2400-3300 cm$^{-1}$. Shifts to the shorter wavenumbers indicate coordination of bismuth with carboxylic acid groups of H$_3$BTC. Thus, according to the results of the XRD and FTIR analysis, Bi-BTC coordination polymer was formed.

The colloidal stability of the as-prepared Bi-BTC frameworks in water was assessed by DLS measurements. The hydrodynamic diameter ($d_{\text{hd}} = 993 \pm 212$ nm) of particles turned out to be approximately twice as high as obtained from the SEM image analysis (figure 2 (a)), so we tried to stabilize Bi-BTC colloid in water. With this aim, we coated Bi-BTC with PAA. After the procedure the hydrodynamic diameter of particles in water decreased to $d_{\text{hd}} = 489 \pm 88$ nm, which indicated the absence of particle aggregation.

Also, a shift of $\xi$ -potential value was observed from $-21.6 \pm 4.8$ mV to $-30.6 \pm 6.7$ mV after the PAA-coating (figure 2 (b)), which confirmed the presence of the negatively charged carboxylic groups of acrylic acid on the particle surface.
Figure 1. (a) SEM image of the Bi-BTC particles; (b) particle size distribution from SEM images (160 individual particles were analysed) fitted with a Gaussian function centred on an average particle diameter; (c) XRD pattern of the Bi-BTC powder; (d) FTIR spectrum of Bi-BTC.

Figure 2. Hydrodynamic diameter (a) and ζ-potential (b) of the nano-sized Bi-BTC particles before and after surface functionalization with PAA.
Then, we examined the X-ray attenuation ability of the prepared nanoparticles. For this aim we used IVIS CT small animal imaging system with 50 kV tube voltage and 1 mA current. The CT contrast intensity continuously increased with increasing particle concentration (figure 3 (a)). Calculated X-ray attenuation linearly depended on the mass concentration of the particles (figure 3 (b)). The slope coefficient was ~2.2 times higher for Bi-BTC (45.9 ± 0.4 HU/mg ml⁻¹) than for barium sulfate (21.2 ± 0.6 HU/mg ml⁻¹), which is a frequently used drug for clinical examinations of the gastrointestinal tract. This result is explained by the higher X-ray mass attenuation coefficient of bismuth element compared to barium [15]. Therefore, using Bi-BTC, lower doses of the drug will be required for obtaining an appropriate contrast in the clinic.

We investigated the ability of Bi-BTC particles to encapsulate cargo molecules. Such dyes as Rose bengal (RB) and Rhodamine B (RhB) are frequently used as fluorescent probes for bioimaging. In addition, RB demonstrated its high antitumor activity as a sono-photosensitizer cancer drug [16]. However, due to the poor pharmacokinetic profile of RB [17] and strong photobleaching of free RhB [18], they may need a stable carrier for delivery to the target tissue in vivo. RB and RhB loading efficiencies to the Bi-BTC nanoparticles were determined by soaking them into the aqueous solutions of the dyes and subsequent comparison of absorption spectra of the dye solution before and after encapsulation at the corresponding peak wavelengths. Successful dye encapsulation was verified by comparing the extinction spectra of free Bi-BTC with those obtained for the dye loaded particles (figure 4). The dye-loaded particles exhibited absorption bands at the characteristic wavelengths of the corresponding dyes (around 560 nm for RB and 554 nm for RhB), which indicated dye adsorption. The LE were 35.5 ± 3.6 % and 17.9 ± 0.6 % for RB and RhB, respectively. The amount of loaded RB by
Bi-BTC is comparable to the Sm-BTC MOF (38 % w/w), which has been reported to be one of the most efficient RB adsorbents among MOFs [19]. The high uptake of BTC-based MOFs may be explained by the π-π stacking between benzene-tricarboxylic acid and RB and multiple hydrogen bonds, whereas interaction with RhB is governed by less strong forces. Nevertheless, Bi-BTC demonstrated the high loading capacity for hydrophilic dyes and can be further used for the biomedical applications which require high local concentrations of therapeutic or diagnostic molecules.

![Figure 4](image_url)

**Figure 4.** Normalized UV-Vis absorption spectra of free Bi-BTC, RB-loaded Bi-BTC and RhB-loaded Bi-BTC frameworks, as well as free dyes in ethanol.

4. Conclusion

In this study we prepared nano-sized Bi-BTC MOFs by microwave synthesis in the presence of a cationic surfactant. The compound was found to be the highly crystalline spherical particles of 455 ± 95 nm diameter with the normal size distribution, which can be stabilized in water using a polyacrylic acid polymer coating. These particles showed higher X-ray enhancement as compared to the conventionally used BaSO₄ contrast and an ability to adsorb sufficiently high amounts of organic dyes. These results reveal promising potential of Bi-BTC complexes in designing theranostic agents for simultaneous CT or fluorescent imaging and delivery of various drug molecules.

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