# ZIF-8 nanoparticles stability in cell culture media

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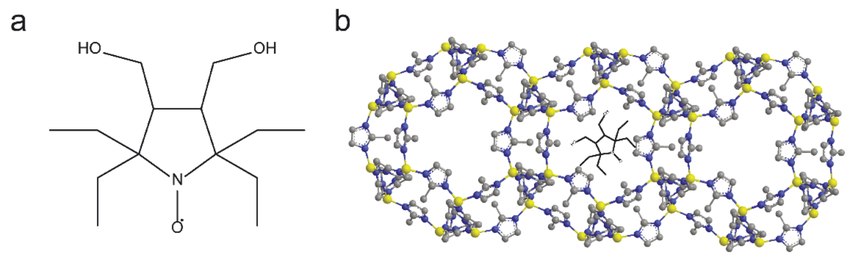
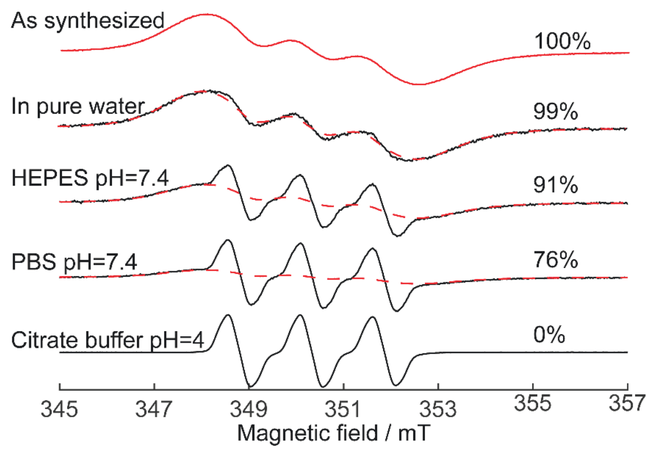
## Introduction

One of the promising platforms for drug delivery is zeolite imidazolate framework-8 (ZIF-8) — metal–organic framework (MOF), formed by the coordination of Zn with 2-methylimidazole ligands. This porous material has several properties convenient for the purpose of drug delivery: a high surface area, adjustable pore size, capability to host a wide range of guests, including photosensitizers, nucleic acids, and proteins. ZIF-8 also exhibits pH-controllable drug release, high efficiency of endosomal escape, and good biocompatibility. The development of drug delivery systems requires in vivo experiments, including studying nanoparticle uptake by cells and cytotoxicity. Cell culture medium is crucial for survival and proliferation of cells, but can affect experimental results and, especially, behavior of the drug delivery platform.




(a) Structure of stable nitroxide radical R used as a guest molecule; (b) ZIF-8 structure with incorporated radical R.

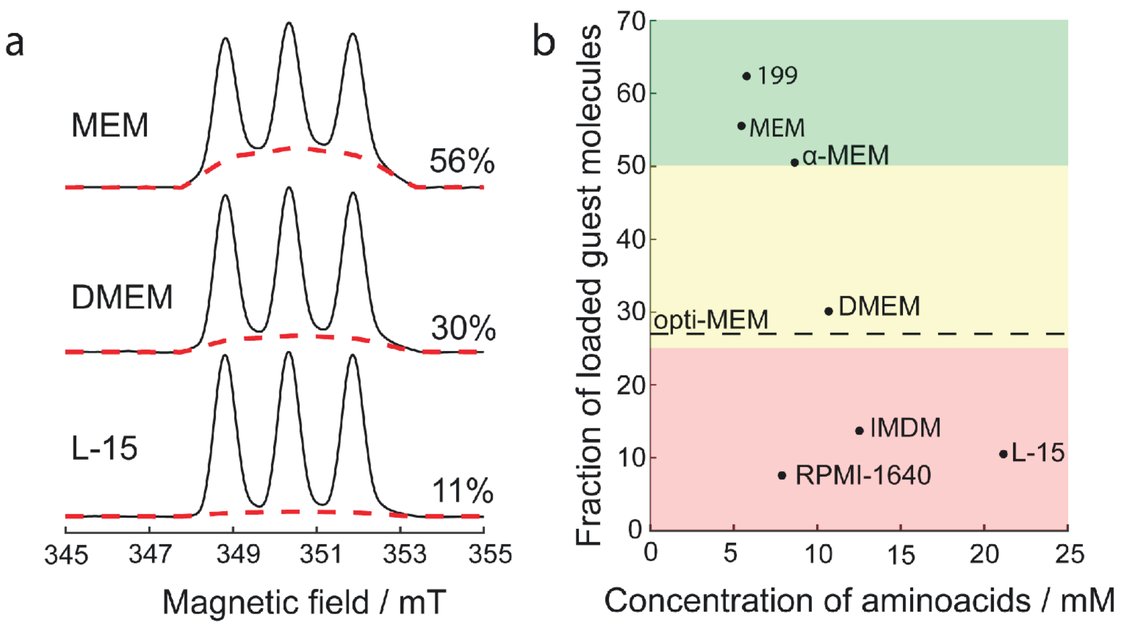
In this work, we studied ZIF-8 nanoparticle's stability in a set of most common cell culture media. We used ZIF-8 nanoparticles containing sterically shielded nitroxide probes with high resistance to reduction and electron paramagnetic resonance (EPR) spectroscopy as a method for quantative analysis. The degradation of ZIF-8 in cell media is accompanied by the cargo leakage, therefore, changes in continuous wave EPR spectrum. We showed that nanoparticles degrade at least partially in all studied media, although the degree of cargo leakage varies widely depending on the medium composition.



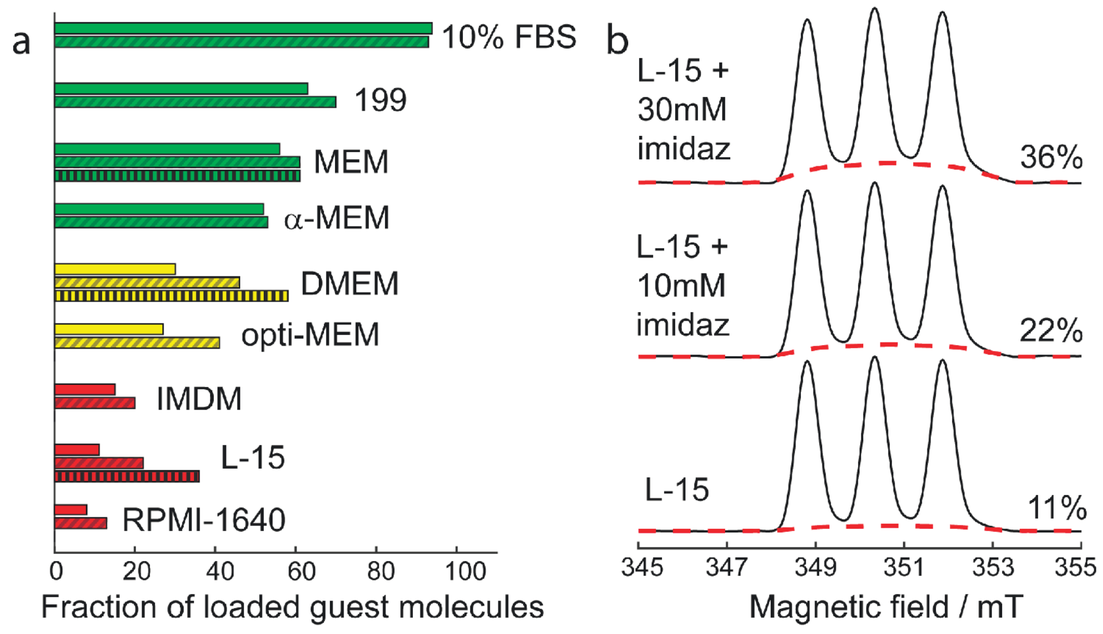

EPR spectra of initial suspension of R@ZIF-8 (as synthesized), R@ZIF-8 5-fold diluted in PBS (5 mM, pH = 7.4), HEPES (5 mM, pH = 7.4), and citrate buffer (0.1 M, pH = 4.0). Red dashed lines show the broad component in the spectra that corresponds to the probes located inside ZIF-8. The number on the right shows the amount of probe remaining inside ZIF-8 after dissolution.

## Cell culture media

We chose the most commonly used cell culture media for our study. All detected spectra contained three noticeable narrow lines (free radical in the solution) demonstrating considerable decomposition of ZIF-8 nanoparticles. There are at least two contributions to ZIF-8 destabilization in these media: dissolution by amino acids and buffer components. One of the possible degradation mechanisms transpires due to the binding of amino acids with zinc leading to release $Zn^{2+}$ and 2-methylimidazole ligands from ZIF-8 nanoparticles. Therefore, we analyzed how the total concentration of amino acids in the media correlates to the extent of ZIF-8 degradation. Figure on the left shows the tendency towards a decreasing R@ZIF-8 amount with increased amino acid concentrations. Presumably, the preliminary addition of 2-methylimidazole into cell media can shift equilibrium state with $Zn^{2+}$ and amino acids and suppress the dissolution of ZIF-8 nanoparticles. Our results demonstrated that 2-methylimidazole inhibited the dissolution of ZIF-8 by amino acids.




Dissolution of ZIF-8 in cell culture media. (a) First integrals of CW EPR spectra of R@ZIF-8 in culture media. Red dashed lines show the spectra obtained by simulation for a fraction of the probe inside ZIF-8. The numbers on the right demonstrate the weight of this fraction; (b) the fraction of probes inside ZIF-8 versus total amino acids concentration in studied media. Stability is denoted by color-coding: red—most nanoparticles dissolved, yellow—middle situation, and green—half or more nanoparticles intact. The result for opti-MEM is demonstrated as a dotted line, because its complete composition and concentration of amino acids are confidential.

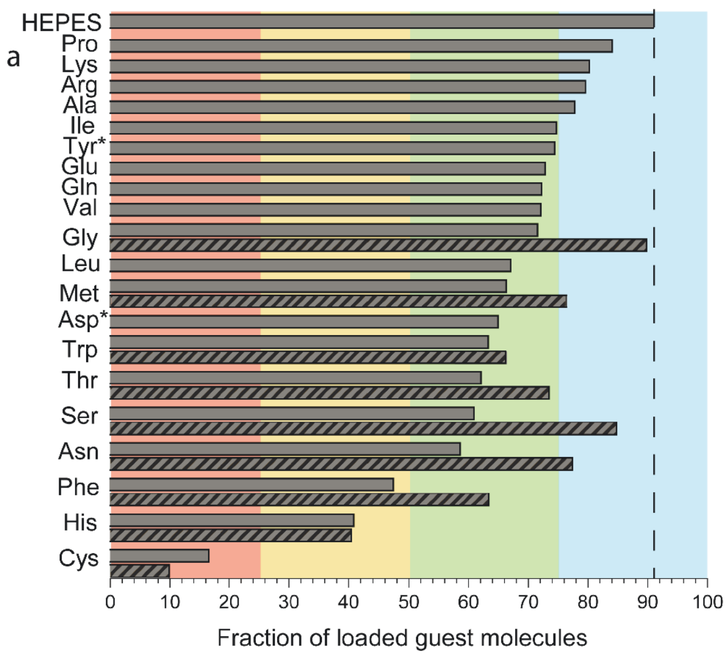


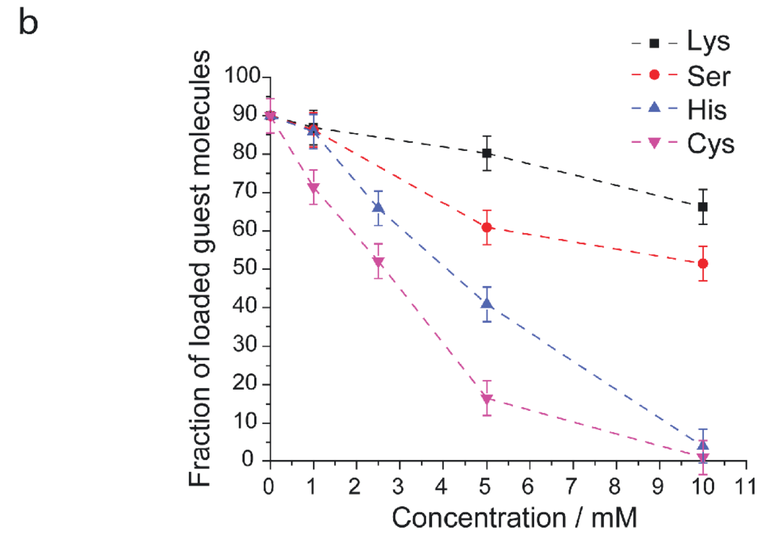

(a) Changes in fraction of probe inside ZIF-8, upon addition of different cell media. Oblique hatching marks samples with addition of 10 mM 2-methylimidazole, vertical hatching—addition of 30 mM 2-methylimidazole; (b) first integrals of CW EPR spectra for R@ZIF-8 with addition of L-15 and MEM. Red dashed lines show the fraction of radicals remaining in ZIF-8; numbers on the right demonstrate the weight of this fraction.

## Amino acids

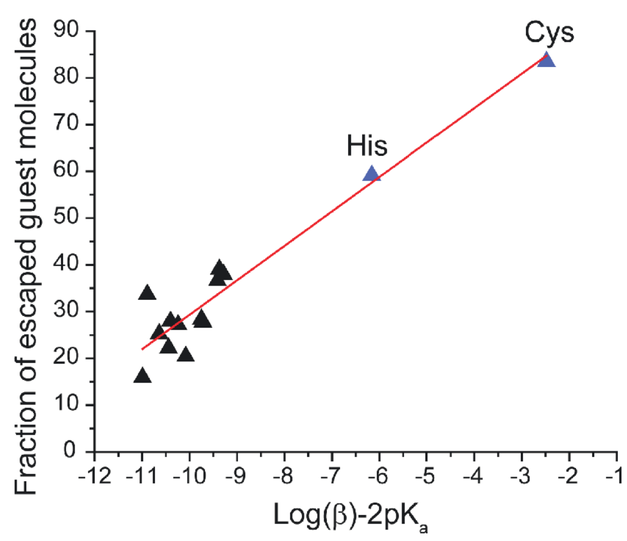
For better understanding of the the role of different amino acids in ZIF-8 degradation, we investigated EPR spectra of R@ZIF-8 five-fold diluted in HEPES 5 mM containing 5 mM of individual amino acids. The dissolution of R@ZIF-8 in the presence of 5 mM of proline, lysine, arginine, and alanine induced only a slight cargo release, relative to the value in HEPES. However, for cysteine almost all probes escaped at the same concentrations meaning full degradation of ZIF-8. Similarly, a significant degradation of nanoparticles ocurred in the presence of histidine and phenylalanine.

We also measured the concentration dependence of the released probes for several amino acids. The number of released probes rose upon increasing amino acid concentrations in all cases., but it transpired considerably faster for cysteine and histidine than for lysine and serine. We assumed that the degradation of nanoparticles is caused by the binding of zinc ions with the amino acids. The stability constants (β) describe the following model reaction: $Zn^{2+} + 2AA \rightarrow Zn(AA)_2$, where AA represents an amino acid in deprotonated form. We also considered that the amino acids have different dissociation degrees at the used pH = 7.4, depending on the acidity constant $K_a$. Hence, the zinc complexation in amino acid solutions depends on two constants: β and Ka. Thus, we examined the relationship between ZIF-8 degradation and log(βKa2) in amino acid solutions.




(a) Fraction of the probe inside ZIF-8 in R@ZIF-8 sample 5-fold diluted in HEPES (5 mM, pH = 7.4) containing 5 mM of individual amino acids. Hatching demonstrates samples with 10 mM 2-methylimidazole, preliminary added to media. * marks two amino acids with different concentrations—the highest we achieved considering the solubility of these amino acids in water: Asp - 2.5 mM, Tyr - 1 mM; (b)—Dependence of the probe inside ZIF-8 on amino acids concentration added.




Correlation between a fraction of spin probes escaped from ZIF-8 and factor Log(β)-2pKa, being a combination of stability constant with zinc and pKa for amino acids.

## Conclusion

We demonstrated that the ZIF-8 nanoparticles degrade to some degree in all of the studied culture media, although the amount of escaped cargo varies widely, depending on composition of cell media. Two main factors led to degradation: the total concentration of amino acids in the media and the buffer. Increase in amino acids concentration caused more cargo leakage, and media with a high PBS or HEPES concentration, namely RPMI-1640 and IMDM, showed high pecentage of escaped guest molecules. We also established the role of individual amino acids in ZIF-8 dissolution: the amount of cargo escaped from ZIF-8 depends on the stability constants for the amino acids with zinc ions. Moreover, 2-methylimidazole partially inhibited ZIF-8 dissolution in several media. This work was supported by the Russian Science Foundation (20-73-10239).

[1] Spitsyna, A.S.; Poryvaev, A.S.; Sannikova, N.E.; Yazikova, A.A.; Kirilyuk, I.A.; Dobrynin, S.A.; Chinak, O.A.; Fedin, M.V.; Krumkacheva, O.A. Stability of ZIF-8 Nanoparticles in Most Common Cell Culture Media, *Molecules* 2022, 27, 3240, DOI: 10.3390/molecules27103240