Available online at www.sciencedirect.com
ScienceDirect
Mendeleev Commun., 2018, **28**, 73–75

Mendeleev Communications

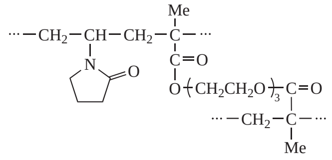
New nitric oxide-carrier systems based on an amphiphilic copolymer of *N*-vinylpyrrolidone with triethylene glycol dimethacrylate

Svetlana V. Kurmaz,* Tatiana N. Rudneva* and Nataliya A. Sanina

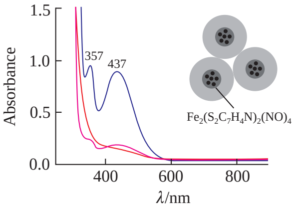
Institute of Problems of Chemical Physics, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation. Fax: +7 496 522 3507; e-mail: skurmaz@icp.ac.ru, ruta@icp.ac.ru

DOI: 10.1016/j.mencom.2018.01.024

The polymer nanoparticles based on an amphiphilic copolymer of *N*-vinylpyrrolidone with triethylene glycol dimethacrylate downloaded by antitumor agents (binuclear nitrosyl complexes of iron) were produced.



Branched VP-TEGDM copolymer



The development of new nitric oxide-carrier systems based on biocompatible polymers is an important task. We examined a new class of NO donor nitrosyl complexes of iron with thiol-containing ligands, which are of interest to medical practice.[1–3] Their use is limited due to their low solubility and instability in physiological solutions. To solve these problems, water-soluble polymers [polyvinyl alcohol, poly(meth)acrylic acid, poly-*N*-vinylpyrrolidone (PVP) or collagen[4–6]] were proposed.

Amphiphilic polymers are excellent precursors for the creation of supramolecular structures (micelles, vesicles, membranes, *etc.*) and hybrids based on them.[7–10] The self-organization mechanisms of complex topology polymers are more diverse, and the resulting structures possess unusual properties. Such polymers can be promising for biomedical applications as drug carriers, protein purification, transfection (transfer) genes, antibacterial materials and biomimetics.[7]

Among hydrophilic polymers, PVP has attracted particular attention as a model for natural polymers with amide groups – protein molecules. Linear and crosslinked *N*-vinylpyrrolidone (VP) copolymers are well studied,[11,12] but the branched copolymers have only recently become the subject of detailed investigations.[13–15] The amphiphilic copolymers of VP with triethylene glycol dimethacrylate (TEGDM) were synthesized by free radical copolymerization in solution. Owing to a difference in the reactivity of monomers,[13] the resulting macromolecules consisted mainly of methacrylate units. Growing polymer chains are statistically joined by their second double bond, and irregular branches appear. At the same time, some of the pendant C=C bonds do not participate in the branching reaction, probably, because of steric restrictions. Due to the amphiphilic nature, they exist in polar media as monomolecular micelles and their aggregates (multimolecular micelles), which made it possible to obtain hybrid nanostructures of fullerene C_{60}[16,17] and zinc tetraphenylporphyrinate[18,19] stable in polar media. Thus, branched copolymers may represent an alternative to traditional block copolymers[20] and diphilic PVP with terminal nonpolar groups.[21,22]

The aim of this work was to study the prospects of the amphiphilic VP-TEGDM copolymer with branched topology as a nitric oxide-carrier system for binuclear tetranitrosyl iron complexes $[Fe_2(S_2C_7H_4N)_2(NO)_4]$ (BTz) and $[Fe_2(SC_7H_4N_2)_2(NO)_4]\cdot 2C_3H_6O$ (BIm).

Table 1 Molecular mass characteristics of PVP and VP-TEGDM.

Copolymer	Molecular mass characteristics RI/MALS+RI	
	$M_n \times 10^{-3}$	$M_W \times 10^{-3}$
PVP	12.7/23.3	61.2/68.7
VP-TEGDM	7.7/18.8	41.0/56.4

Complexes were synthesized by published methods[4,23] (for structures, see Figure S1, Online Supplementary Materials). The copolymers of VP with TEGDM and linear PVP were obtained by radical copolymerization in toluene and ethanol,[15] respectively (Table 1).[†] According to IR spectroscopy,[18] the contents of VP and TEGDM units in the copolymer were 0.8 and 0.2 mole fractions, respectively.

To encapsulate BTz and BIm, the VP-TEGDM copolymer solutions (1 mg ml^{-1}) in isopropanol were prepared.[‡] According to DLS, the copolymer of polar (VP) and less polar (methacrylate) units exists in Pr^iOH at 25 °C as monomolecular micelles and their aggregates with the mean hydrodynamic radii of *ca.* 3 and 15 nm, respectively.

According to published data,[2] the absorption spectra of binuclear dinitrosyl complexes with glutathione or cysteine ligands have two intense absorption bands at 310 and 360 nm and a weak band

---

[†] The relative and absolute molecular masses were determined by liquid chromatography on a Waters GPCV 2000 instrument (2 PL-gel columns, 5 μm, MIXED-C, 300×7.5 mm) using refractometer (RI) and light scattering (MALS) detectors.

[‡] The complexes of BTz and BIm were dissolved in hot benzene (1 mg ml^{-1}) to give a yellow-coloured visually transparent solution. Then, 0.1, 0.3 or 0.5 ml of the nitrosyl compound in benzene was added dropwise to 4 ml of the copolymer solution with continuous stirring. The conditions of BTz and BIm encapsulation are given in Tables S1 and S2, see Online Supplementary Materials. Then, organic solvents were evaporated, and 4 ml of an aqueous buffer solution with pH 6.8 was added to the residue. The absorption spectra of the solutions were recorded with a Specord M40 spectrophotometer, the thickness of the cells was 1 or 0.2 cm.

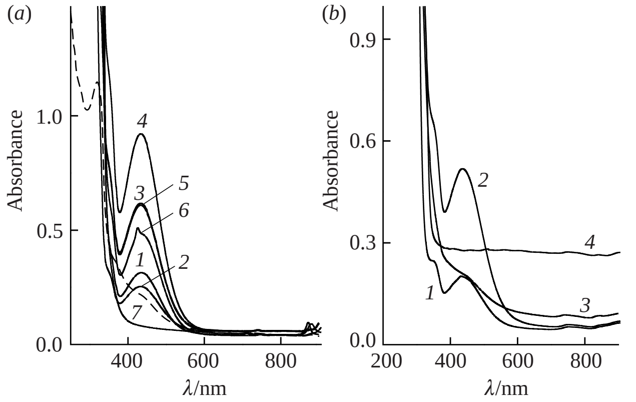


Figure 1 (*a*) Absorption spectra of BTz (*1*) in benzene, (*2*–*6*) in tests 1–5 and (*7*) stored in benzene for 2 months under normal conditions. The dashed curve corresponds to the BTz spectrum in methylene chloride, the cuvette is 0.2 cm. (*b*) Absorption spectra of aqueous buffer solutions of the copolymer/BTz and linear PVP/BTz prepared in (*1*) test 1, (*2*) test 2, (*3*) test 4, and (*4*) test 5.

at 768 nm. Figure 1(*a*) and Figure S2 (see Online Supplementary Materials) show that both BTz and BIm in benzene also reveal specific absorption as a shoulder at ~ 350 nm, and a separate band at 434 or 437 nm, respectively. The absorption bands of these complexes at $\lambda_{\max}$ ~ 320 and 310 nm, which characterize the $\pi \rightarrow \pi^*$ transition of coordinated ligands, were observed in methylene chloride. The theoretical and experimental electronic spectra of binuclear nitrosyl complexes were studied.[24] A band at 430–440 nm is composed of $\pi^*_{\mathrm{NO}} \rightarrow \pi^*_{\mathrm{NO}}$ and $\mathrm{d} \rightarrow \mathrm{d}$ excitations with the participation of sulfur orbitals. Thus, the absorption band at $\lambda_{\max}$ ~ 430 nm could be used as a basis for the evaluation of nitrosyl iron complexes stability in solution.

Figure 1(*a*) shows absorption spectra of BTz in benzene (curve *1*) and solutions prepared in experiments 1–5 (see Table S1, Online Supplementary Materials) in the presence of copolymers (curves *2*–*6*). Spectra of BTz in benzene and in copolymer solutions are similar and spectral parameters of absorption band differ insignificantly despite the different polarity of the media. The spectra identity in experiments 2 and 4 indicates the high stability of the test systems in solutions. The linear dependence of *D* at the maximum of the absorption band of BTz on its concentration in solution testified that intermolecular interactions were absent from the system (Figure S3).

The absorption spectra of solutions during the observation period (over two months) and the electronic structure of the complex remained unchanged. It is assumed that, as a result of the BTz molecule solubilization by polymer particles stable in $\mathrm{Pr^iOH}$, hybrid structures are formed, where a polymer particle serves as a host for guest BTz molecules, and the molecules of the nitrosyl complex are localized in the hydrophobic core of polymer particles. Note that, in a solid state, the complex is very sensitive to storage conditions (as a consequence of its NO-donor properties), and it decomposes quickly at room temperature in air with NO release; upon dissolution, it decomposes to form a precipitate, and its characteristic bands disappear from the absorption spectrum [Figure 1(*a*), curve *7*].

Polymer composites dissolved quickly in aqueous buffer solutions. In experiments 1 and 2, solutions had a yellow colour for about a day and characteristic BTz absorption bands [Figure 1(*b*), curves *1* and *2*]. Compared to $\mathrm{Pr^iOH}$, in aqueous buffer solutions BTz absorption band shifted to longer wavelengths, and its optical density in maximum of absorption band decreased apparently due to the interaction between the copolymer and nitrosyl complex. Thus, an encapsulated complex was stable in aqueous buffer solutions.

However, another aqueous buffer solution of the copolymer with encapsulated BTz (experiments 4 and 5) discoloured within a few minutes; characteristic absorption bands of the complex at $\lambda_{\max}$ ~ 430 nm were absent from the spectrum [Figure 1(*b*), curves *3*, *4*]. This different behaviour of the encapsulated complexes may be due to either decomposition with NO release or bonding with the polymer matrix. The speed and direction of these processes depend on the local environment of BTz. Possibility of nitrosyl complexes binding with proteins, such as albumin, is reported,[25,26] low molecular weight thiol ligands can be replaced by protein fragments through cysteine residue.

According to Figure 2, the hydrodynamic radii R_h at the peak maxima were ~5 and 36 nm for mono- and multimolecular micelles, respectively. However, in an aqueous solution of copolymer/BTz, the value of *I* increased dramatically and the peaks shifted toward higher values of R_h. The main contribution to light scattering at 20 °C was made by particles with R_h ~ 100 nm. At 40 °C, the value of *I* increased to about 3×10^5 and the average particle size reduced to about 80 nm.

The copolymer/BTz (experiment 2) was isolated from aqueous solutions, dried in air, and studied by IR spectroscopy (Figure S4, see Online Supplementary Materials). The strong and characteristic absorption bands at 1724 and 1672 cm^{-1} (related to the stretching C=O vibrations of VP and TEGDM units) of copolymer were observed in IR spectrum. Despite the high content of BTz (7.5 wt% on a copolymer basis) in the sample, its most intense absorption bands at 1721 and 1787 cm^{-1} corresponding to vibrations of groups N=O were not detected. Moreover, an absorption band at 1724 cm^{-1} related to the stretching vibrations of C=O bands in methacrylic group of TEGDM units of the copolymer was almost disappearing and a number of uncharacteristic bands of the copolymer at 700–1200 cm^{-1} emerged. These spectral changes, apparently, serve as evidence of the nonvalent binding of BTz with the copolymer in the solid state and point to its localization in the hydrophobic core of polymer particles consisting of dimethacrylate fragments. Similar results were also obtained for the BIm complex (see Online Supplementary Materials).

An amperometric study[§] of the solutions and films of copolymer with encapsulated BTz and BIm complexes showed no signals due to nitric oxide release.

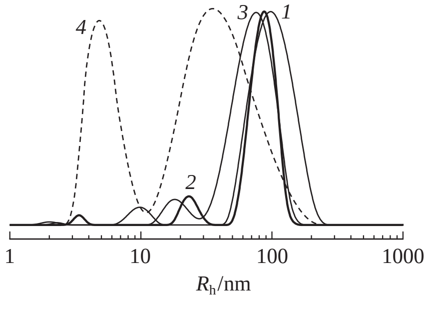


Figure 2 Light scattering intensity distribution on copolymer/BTz particle sizes in water buffer solution at (*1*) 20, (*2*) 30 and (*3*) 40 °C. The dashed curve *4* corresponds to the copolymer solution at 20 °C.

The particle size distribution in the buffer solution was studied by DLS. The solutions were filtered through a filter with a pore diameter of 0.45 μm. The vials with the solution were thermostated at 20 °C for ~0.5 h. DLS measurements were carried out at a detection angle of 90° using a Photocor Compact instrument equipped with a laser diode (654 nm). The experimental data were processed using the DynaLS v. 2.8.3 software.

The IR spectra of the copolymer and powdered polymer composites were recorded on a FTIR Bruker ALPHA spectrophotometer in a range of 4000–400 cm^{-1} in the ATR mode.

[§] The 'amiNO-700' sensor electrode for inNO Nitric Oxide Measuring System (Innovative Instruments) was used.

Thus, we have demonstrated for the first time that the binuclear tetranitrosyl iron complexes of the μ-S–C–N structural type can be encapsulated in micellar polymeric nanoparticles of amphiphilic copolymer VP-TEGDM. As a result of solubilization of BTz or BIm molecules by the polymeric particles, hybrid structures stable in aqueous buffer solutions are formed; guest molecules of the complex localized in the hydrophobic nucleus bind noncovalently to C=O groups of dimethacrylate units of polymer chains. The copolymer is biocompatible and penetrates into the cells;[18] the results of this work are promising for biomedical practices.

This work was supported by the Russian Foundation for Basic Research (grant no. 16-33-00592).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2018.01.024.

References

1 A. F. Vanin, *Dinitrozil'nye kompleksy zheleza s tiolsoderzhashchimi ligandami. Fizikokhimiya, biologiya, meditsina* (*Dinitrosyl Iron Complexes with Thiol Containing Ligands. Physical Chemistry, Biology, Medicine*), IKI, Izhevsk, 2015 (in Russian).

2 W. A. Wani, U. Baig, S. Shreaz, R. A. Shiekh, P. F. Iqbal, E. Jameel, A. Ahmad, S. H. Mohd-Setapar, M. Mushtaque and L. T. Hun, *New J. Chem.*, 2016, **40**, 1063.

3 N. A. Sanina, O. S. Zhukova, Z. S. Smirnova, T. N. Rudneva, G. V. Shilov, S. M. Aldoshin and M. I. Davydov, *Patent of India 268939*, 2015.

4 N. Yu. Shmatko, N. A. Sanina, D. V. Anokhin, A. A. Piryazev, D. A. Ivanov, A. V. Kulikov and S. M. Aldoshin, *Russ. Chem. Bull., Int. Ed.*, 2015, **64**, 1616 (*Izv. Akad. Nauk, Ser. Khim.*, 2015, 1616).

5 A. B. Shekhter, T. G. Rudenko, L. P. Istranov, A. E. Guller, R. R. Borodulin and A. F. Vanin, *Eur. J. Pharm. Sci.*, 2015, **78**, 8.

6 Y. Wo, E. J. Brisbois, R. H. Bartlett and M. E. Meyerhoff, *Biomater. Sci.*, 2016, **4**, 1161.

7 Y. Zhou and D. Yan, *Chem. Commun.*, 2009, 1172.

8 Y. Zhou, W. Huang, J. Liu, X. Zhu and D. Yan, *Adv. Mater.*, 2010, **22**, 4567.

9 L. Ya. Zakharova, R. R. Kashapov, T. N. Pashirova, A. B. Mirgorodskaya and O. G. Sinyashin, *Mendeleev Commun.*, 2016, **26**, 457.

10 A. A. Vdovchenko, A. V. Hubina, E. G. Vlakh and T. B. Tennikova, *Mendeleev Commun.*, 2017, **27**, 153.

11 F. P. Sidel'kovskaya, *Khimiya* N-*vinilpirrolidona i ego polimerov (Chemistry of* N-*Vinylpyrrolidone and its Polymers)*, Nauka, Moscow, 1970 (in Russian).

12 Yu. E. Kirsh, *Poli*-N-*vinilpirrolidon i drugie poli*-N-*vinilamidy* [*Poly-(*N-*vinylpyrrolidone) and other Poly(N-vinylamides)*], Nauka, Moscow, 1998 (in Russian).

13 S. V. Kurmaz and A. N. Pyryaev, *Polym. Sci., Ser. B*, 2010, **52**, 5.

14 S. V. Kurmaz and A. N. Pyryaev, *Mendeleev Commun.*, 2010, **20**, 52.

15 S. V. Kurmaz and A. N. Pyryaev, *Russ. J. Gen. Chem.*, 2012, **82**, 1705 (*Zh. Obshch. Khim.*, 2012, **82**, 1700).

16 S. V. Kurmaz and N. A. Obraztsova, *Mendeleev Commun.*, 2015, **25**, 350.

17 S. V. Kurmaz, N. A. Obraztsova, E. O. Perepelitsina, G. V. Shilov, D. V. Anokhin and E. V. Pechnikova, *Mater. Today Commun.*, 2015, **4**, 130.

18 S. V. Kurmaz, N. A. Obraztsova, A. A. Balakina and A. A. Terent'ev, *Russ. Chem. Bull., Int. Ed.*, 2016, **65**, 2097 (*Izv. Akad. Nauk, Ser. Khim.*, 2016, 2097).

19 S. V. Kurmaz, V. Yu. Gak, V. A. Kurmaz and D. V. Konev, *Russ. J. Phys. Chem. A*, 2018, **92**, 329 (*Zh. Fiz. Khim.*, 2018, **92**, 293).

20 V. P. Torchilin, *Adv. Drug Deliv. Rev.*, 2012, **64**, 302.

21 O. V. Klimenko and M. I. Shtilman, *Cancer Gene Ther.*, 2013, **20**, 237.

22 A. N. Kuskov, A. A. Voskresenskaya, A. V. Goryachaya, A. A. Artyukhov, M. I. Shtilman and A. M. Tsatsakis, *J. Mater. Sci. Mater. Med.*, 2010, **21**, 1521.

23 N. Sanina, T. Roudneva, G. Shilov, R. Morgunov, N. Ovanesyan and S. Aldoshin, *Dalton Trans.*, 2009, 1703.

24 M. Jaworska and Z. Stasicka, *New J. Chem.*, 2005, **29**, 604.

25 T. Roudneva, N. Sanina, D. Mischenko, E. Frog, R. Kotelnikova and S. Aldoshin, *Nitric Oxide*, 2008, **19**, S43.

26 L. A. Syrtsova, N. A. Sanina, E. N. Kabachkov, N. I. Shkondina, A. I. Kotelnikov and S. M. Aldoshin, *RSC Adv.*, 2014, **4**, 24560.

Received: 20th February 2017; Com. 17/5182