Available online at www.sciencedirect.com
ScienceDirect
Mendeleev Commun., 2022, **32**, 117–119
Mendeleev Communications

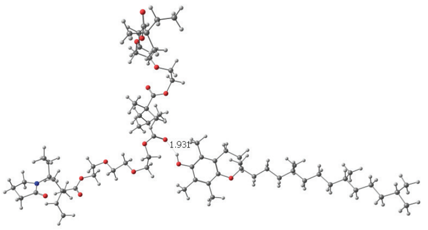
# New water-soluble forms of $\alpha$-tocopherol: preparation and study of antioxidant activity *in vitro*

**Svetlana V. Kurmaz,*[a] Natalia V. Fadeeva,[a] Julia A. Skripets,[b] Roman I. Komendant,[a] Vladislav M. Ignatiev,[a,b] Nina S. Emel’yanova,[a] Yuliya V. Soldatova,[a] Irina I. Faingold,[a] Darya A. Poletaeva[a] and Raisa A. Kotelnikova[a]**

[a] *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*
[b] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation*

DOI: 10.1016/j.mencom.2022.01.038

**Water-soluble forms of $\alpha$-tocopherol (vitamin E) possessing antioxidant activity *in vitro* were obtained by encapsulating in *N*-vinylpyrrolidone with triethylene glycol dimethacrylate polymer particles and were characterized by various physicochemical methods. Quantum-chemical modeling of a structure of $\alpha$-tocopherol with the copolymer moiety and its theoretical absorption spectra modeling were carried out.**



*Keywords*: *N*-vinylpyrrolidone, triethylene glycol dimethacrylate, amphiphilic copolymer, $\alpha$-tocopherol, encapsulation, antioxidant activity.

(±)-$\alpha$-Tocopherol (TP) is one of the highly bioactive fat-soluble vitamins from the E family of tocopherols and tocotrienols.[1] Lipophilic TP molecules are able to inactivate free radicals directly in the hydrophobic layer of membranes owing to a hydrogen atom transfer to the free radical of lipid peroxide, thus preventing the development of the lipid peroxidation chain (LPO).[2,3] The research on the antioxidant properties of TP new dosage forms is an urgent task in biomedical chemistry, since free radical reactions are involved in the pathogenesis of many diseases.[4] However, their intravenous application in the treatment of pathological conditions[5–8] is limited because of the low solubility of TP in water. Amphiphilic polymer carriers increase the hydrophilicity of drugs with low solubility in water and raise their potential for biomedical applications. Polymeric carriers of the micellar type on the basis of the amphiphilic block copolymers, liposomes, *etc*., with encapsulated hydrophobic drugs demonstrate high efficiency,[9–15] given that they can circulate in the bloodstream for a long time, accumulate in tissues with increased permeability (tumors) and gradually release the drug. Our early works[16–20] have shown that *N*-vinylpyrrolidone (VP) based amphiphilic copolymers with branches in polymer chains may be of interest as modern platforms for lipophilic compounds: zinc tetraphenylporphyrinate, nitrosyl iron complexes, platinum(IV) complexes, and fullerene. They exhibit low cytotoxicity and are able to penetrate cells *in vitro* to deliver the active substance.[20]

The aims of this work were to obtain a water-soluble form of TP by its encapsulating in polymer particles of VP with triethylene glycol dimethacrylate (TEGDM) and to explore its antioxidant activity *in vitro*.

The amphiphilic copolymer was obtained by radical copolymerization in toluene from the monomer mixture [VP] : [TEGDM] with the molar ratio of 100 : 2, according to the published procedure.[20] Its synthesis, reaction mixture isolation details and chemical structure are presented in Online Supplementary Materials. The main physicochemical characteristics of the copolymer are given in Table 1. Monomer unit composition of the copolymer was calculated from the data of elemental analysis. The values of the absolute molecular weight $M_w$ of the scattering centers, the second virial coefficient and hydrodynamic radii of scattering centers were estimated from the data of dynamic light scattering by water copolymer solution at 22 °C. Above the critical aggregation concentration (CAC) of the copolymer in water, the solution contains scattering centers of different size (Table 1). There is an assumption that they correspond to the individual macromolecules and their aggregates. The small size of macromolecules may be caused by branched character of polymer chains and high density of their molecular packaging. In addition, the hydration shell of water molecules[21] promotes the convergence of the pyrrolidone rings of the polymer chain moieties consisting of VP units, and a change in their conformation as in the case of linear polyvinylpyrrolidone. Macromolecules compising polar and low-polarity fragments are inherently micellar.[20]

The conditions for TP encapsulating and concentration of reagents in $\mathrm{Pr^iOH}$ and TP–polymer nanostructures in water are presented in Tables S1–S3 (Online Supplementary Materials). TP was encapsulated using the copolymer solutions in $\mathrm{Pr^iOH}$ with various concentrations. The average value of the hydrodynamic radius of the scattering centers $R_h$ in $\mathrm{Pr^iOH}$ was ~4 nm and has not changed with increasing temperature. TP concentrations varied from $2.7 \times 10^{-3}$ to $1.3 \times 10^{-4}$ mol dm$^{-3}$.

It is known,[22] that TP hydroxyl group can participate in the formation of intermolecular hydrogen bonds. The formation of

**Table 1** Physicochemical characteristics of the VP–TEGDM copolymer.

| Copolymer | [VP] : [TEGDM]/ mol% | $M_w \times 10^{-4}$/ g mol$^{-1}$ | CAC in water/ mg ml$^{-1}$ | $R_h$[a] in water/ nm |
|---|---|---|---|---|
| VP–TEGDM | 94.3 : 5.7 | 47.0 | 2.8 | 4; 77 |

[a] Measured at 2.5 mg ml$^{-1}$ copolymer concentration in water.

intermolecular H-bonds has been proved by the broad absorption band of stretching vibrations of OH group in the region of 3700–3100 cm$^{-1}$ in its IR spectrum (see Online Supplementary Materials). Meanwhile, some of OH groups of TP remain free, as evidenced by the narrow absorption band at 3630 cm$^{-1}$. Stretching vibrations of the C=C bond of the aromatic nucleus are observed in the range of 1610–1580 cm$^{-1}$. The characteristic absorption bands at 1097 and 813 cm$^{-1}$ refer to the tetrahydropyran cycle, and the absorption band at 1169 cm$^{-1}$ relates to the tocol fragment.[22] The absorption intensity of the band at 290 nm depends linearly on TP concentration in $Pr^iOH$ and obeys the Beer's law, which reveals the absence of any intermolecular interactions between the components of solution.

TP is practically insoluble in water, therefore its absorption band has not been revealed in the range of 280–300 nm in the spectra of aqueous solutions of control samples not containing copolymer. Meanwhile, the TP absorption band at 290 nm was observed in the spectra of copolymer aqueous solutions as a result of its solubilization by the amphiphilic copolymer and an increase in solubility [Figure 1(*a*)]. Its broadening and asymmetric shape is apparently due to TP localization in the regions of the polymer micelle having different polarity. Thus, TP–copolymer structures are formed in an aqueous solution, when molecules with the van der Waals volume of about 1.9 nm$^3$ (the calculation was made on the basis of optimized molecule geometry) occupy the internal cavities of the polymer particle. The TP content per copolymer reaches ~10 wt%.

The effective binding constant $K_{ef}$ of TP with copolymer determined as described[23] was 0.72 × 10$^4$ dm$^3$ mol$^{-1}$ at 22 °C under encapsulating conditions (see Table S1).

The same features were observed in the absorption spectra of aqueous solutions of $TP_{sol}$ encapsulated in copolymer solutions with concentrations of 3 and 7 mg ml$^{-1}$. However, the TP absorption band shifted towards longer wavelengths, with maximum occurred at 298 nm. That indicates the localization of TP molecules mainly in polar segments of polymer particles. The absorption in visible region became pronounced because of the high concentration of the copolymer in solution.

Quantum-chemical modeling demonstrated that TP molecules were capable of forming hydrogen bonds with electron donor groups of TEGDM units of the hydrophobic core of the polymer particle (see Online Supplementary Materials). Thus, the length of the hydrogen bond between the OH group of TP and the C=O group of the TEGDM unit was 1.931 Å (tpssh/6–31G*), that indicated its sufficient strength. The TP orientation relative to the hydrophobic brush of TEGDM suggests the possibility of attachment of several TP molecules to this polymer moiety.

TDDFT modeling of theoretical absorption spectra of the TP–copolymer moiety complex in aqueous solutions was carried out [Figure 1(*b*)]. The shape of the theoretical spectra tallies well with the experimental results. However, the maximum of the main absorption band in the theoretical TP spectrum refers to 275 nm; *i.e.*, it slightly shifts to shorter wavelengths in comparison with the experimental data. At the same time, it completely coincides with the value obtained in the earlier work.[24] This band is characterized by only one electron $\pi \rightarrow \pi^*$ transition in the aromatic ring of TP. In the theoretical TP spectrum, the main band of the spectrum of TP–copolymer moiety complex shifts to the short wavelength region for 5 nm more and its intensity increases. This is due to contributions of not only $\pi \rightarrow \pi^*$ transition of TP, but also transitions from the HOMO TP to $\pi^*$ orbital of the copolymer moiety. That can also explain the increase of the intensity of the main band in the experimental spectrum.

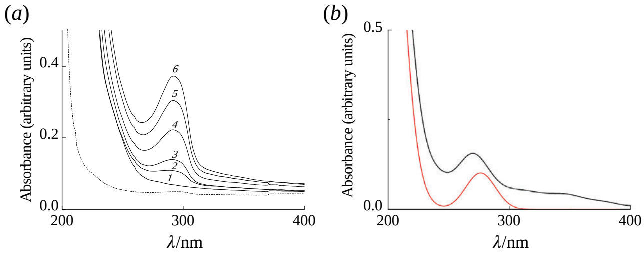


**Figure 1** (*a*) Absorption spectra of aqueous buffer solution (pH 7.4) of encapsulated TP in polymer particles: [copolymer] = 0.35 mg ml$^{-1}$ and [TP] = 0.057 mg ml$^{-1}$; (*b*) theoretical spectra (tpssh/6–31G*) of the TP with the VP–TEGDM–TEGDM polymer moiety (black) and TP (red). Curve numbers refer to the data in Table S1. The dotted line shows the spectrum of water buffer.

According to DLS data, the light scattering intensity *I* increased significantly in aqueous buffer solutions of the copolymer with encapsulated TP (0.5–1%), and the distribution curves of light scattering intensity *vs.* the sizes of scattering centers were different from those of the initial copolymer (see Online Supplementary Materials). At room temperature, the distribution of the light scattering intensity over the sizes of the scattering centers of the copolymer was bimodal, and the solution contained particles with hydrodynamic radii of ~4 and 120 nm. It becomes narrower in an aqueous solution of polymer particles loaded with TF owing to their self-organization and a change in the solution structure. The main contribution to light scattering is made by particles with a peak size of about 80–90 nm. The studied systems, like the copolymer, were thermally sensitive, as evidenced by the temperature dependence of *I* and $R_h$. The hydrogen bond between the C=O groups of the VP lactam cycle and water is obviously destroyed at temperatures above 35 °C and the polar shell collapsed. As a result, the solubility of polymer particles with encapsulated TP decreases. The release of the active substance from polymer particles is possible due to the destruction of the hydrogen bond between the copolymer and TP caused by temperature effect and the transition of the macromolecule from the coil-globule state.

To study the antioxidant activity, the copolymer composition with encapsulated TP content of ~3.7% was obtained. Its IR spectrum shows mainly absorption bands of the copolymer, in particular, an intense absorption band of stretching vibrations of the C=O group of the lactam cycle of VP units at 1651 cm$^{-1}$, which forms the hydrogen bond with water. The shoulder at 1721 cm$^{-1}$ was attributed to C=O oscillations of the TEGDM group. Main TP absorption bands were not found in the polymer composition, apparently because of its low content. Scattering centers with $R_h$ ~50 nm were revealed in an aqueous buffer solution at TP–copolymer composition concentration of 3 mg ml$^{-1}$. When the solution was diluted, unimodal particles with $R_h$ ~40 nm were observed only in the solution.

In this work, the antioxidant activity of a new water-soluble form of TP was assessed by measuring the intensity of lipid peroxidation in a homogenate of mouse brain tissue *in vitro*.[25,26] The intensity of lipid peroxidation was determined by the content of malondialdehyde (MDA) in the samples, which is one of the end products of lipid peroxidation forming in the mouse brain homogenate. The rate of MDA formation allows one to estimate the activation or inhibition of the LPO process. It has been shown that $TP_{sol}$ slows down the accumulation of MDA and exhibits antioxidant properties. Thus, it inhibits LPO *in vitro* by 37 and 51% at concentrations of 5 × 10$^{-4}$ and 10$^{-3}$ M, respectively (see Online Supplementary Materials).

Kinetic curves of MDA accumulation during spontaneous LPO reaction and in the presence of $TP_{sol}$ (TP concentration of 10$^{-3}$ M) are shown in Figure 2. As can be seen, $TP_{sol}$ effectively reduces the rate of MDA accumulation. Comparison of the results obtained under similar conditions reveals that the antioxidant activity of $TP_{sol}$ is determined by tocopherol action.

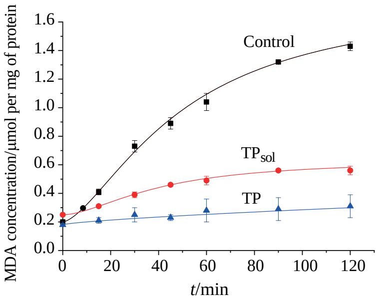


**Figure 2** The rate of MDA accumulation in the mouse brain homogenate under the action of TP$_{sol}$ and TP ($10^{-3}$ M). Control sample – mouse brain homogenate free of the test compounds. Results were expressed as mean ± standard error of the mean. Significance of differences was assessed by the Student's *t*-test. The differences were significant at $p < 0.001$.

In conclusion, new water-soluble forms of the α-tocopherol containing up to 10 wt% of the active substance (calculated on the copolymer) were obtained based on the amphiphilic copolymer of *N*-vinylpyrrolidone with triethylene glycol dimethacrylate. The encapsulated TP was found to possess *in vitro* antioxidant activity and ability to slow down the accumulation of malondialdehyde. Thus, these compounds may be promising for intravenous administration in the treatment of various pathological conditions.

The work was performed on state assignment, state registration nos. AAAA-A19-119041090087-4 and AAAA-A19-119071890015-6.

*Online Supplementary Materials*

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2022.01.038.

## References

1 N. Duhem, F. Danhier and V. Préat, *J. Controlled Release*, 2014, **182**, 33.
2 B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford, 2015.
3 J.-F. Poon and D. A. Pratt, *Acc. Chem. Res.*, 2018, **51**, 1996.
4 B. Halliwell, *Nutr. Rev.*, 2012, **70**, 257.
5 Yu. N. Schanin, V. Yu. Schanin and E. V. Zinovyev, *Antioksidantnaya terapiya v klinicheskoi praktike* (*Antioxidant Therapy in Clinical Practice*), ELBI-SPb, St. Petersburg, 2003 (in Russian).
6 M. G. Traber, T. D. Schiano, A. C. Steephen, H. J. Kayden and M. Shike, *Am. J. Clin. Nutr.*, 1994, **59**, 1270.
7 P. García, J. Vega, P. Jimenez, J. Santos and P. Robert, *Eur. J. Lipid Sci. Technol.*, 2013, **115**, 811.
8 Y. Yang and D. J. McClements, *Food Chemistry*, 2013, **141**, 473.
9 *Nanoparticulates as Drug Carriers*, ed. V. P. Torchilin, Imperial College Press, London, 2006.
10 G. Kwon and M. Forrest, *Drug Dev. Res.*, 2006, **67**, 15.
11 K. Letchford and H. Burt, *Eur. J. Pharm. Biopharm.*, 2007, **65**, 259.
12 G. S. Kwon and M. L. Forrest, *US Patent 8173167:B2*, 2012.
13 H. Tabandeh and S. A. Mortazavi, *Iran. J. Pharm. Res.*, 2013, **12**, S21.
14 I. G. Zigoneanu, C. E. Astete and C. M. Sabliov, *Nanotechnology*, 2008, **19**, 105606.
15 N. Varga, Á. Turcsányi, V. Hornok and E. Csapó, *Pharmaceutics*, 2019, **11**, 357.
16 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).
17 S. V. Kurmaz, T. N. Rudneva and N. A. Sanina, *Mendeleev Commun.*, 2018, **28**, 73.
18 S. V. Kurmaz, N. V. Fadeeva, B. S. Fedorov, G. I. Kozub, N. S. Emel'yanova, V. A. Kurmaz, R. A. Manzhos, A. A. Balakina and A. A. Terentyev, *Mendeleev Commun.*, 2020, **30**, 22.
19 S. V. Kurmaz, V. D. Sen', A. V. Kulikov, D. V. Konev, V. A. Kurmaz, A. A. Balakina and A. A. Terentyev, *Russ. Chem. Bull., Int. Ed.*, 2019, **68**, 1769 (*Izv. Akad. Nauk, Ser. Khim.*, 2019, 1769).
20 S. V. Kurmaz, N. A. Obraztsova, A. A. Balakina and A. A. Terentyev, *Russ. Chem. Bull., Int. Ed.*, 2016, **65**, 2097 (*Izv. Akad. Nauk, Ser. Khim.*, 2016, 2097).
21 S. V. Kurmaz, N. V. Fadeeva, V. M. Ignat'ev, V. A. Kurmaz, S. A. Kurochkin and N. S. Emel'yanova, *Molecules*, 2020, **25**, 6015.
22 O. V. Checheta, E. F. Safonova and A. I. Slivkin, *Vestnik VGU, Ser. Khim., Biol., Farmatsiya*, 2010, no. 2, 164 (in Russian).
23 K. A. Connors, *Binding Constants: The Measurement of Molecular Complex Stability*, John Wiley & Sons, Inc., New York, 1987.
24 G. Singh, R. Sachdeva, B. Rai and G. S. S. Saini, *J. Mol. Struct.*, 2017, **1144**, 347.
25 T. F. Slater, *Methods Enzymol.*, 1984, **105**, 283.
26 H. Ohkawa, N. Ohishi and K. Yagi, *Anal. Biochem.*, 1979, **95**, 351.

*Received: 27th May 2021; Com. 21/6569*