ISSN 1560-0904, Polymer Science, Series B, 2023, Vol. 65, No. 2, pp. 129–132. © Pleiades Publishing, Ltd., 2023.
ISSN 1560-0904, Polymer Science, Series B, 2023. © Pleiades Publishing, Ltd., 2023.
Russian Text © The Author(s), 2023, published in Vysokomolekulyarnye Soedineniya, Seriya B, 2023, Vol. 65, No. 2, pp. 117–121.

MEDICAL POLYMERS

# Copolymers of Sodium Styrene Sulfonate and 2-Deoxy-2-methacrylamido-D-glucose with Antiviral Activity

**E. F. Panarin[a,*], O. N. Zhuravskaya[a], and A. A. Shtro[b]**

[a] *Institute of Macromolecular Compounds, Russian Academy of Sciences, St. Petersburg, 199004 Russia*

[b] *Smorodintsev Influenza Research Institute, St. Petersburg, 197376 Russia*

**e-mail: efra99@mail.ru*

Received November 15, 2022; revised December 1, 2022; accepted December 29, 2022

**Abstract**—The radical copolymerization of sodium styrene sulfonate with vinyl saccharide 2-deoxy-2-methacrylamido-D-glucose in water is studied. For sodium styrene sulfonate and 2-deoxy-2-methacrylamido-D-glucose the reactivity ratios are found to be $r_1 = 1.58 \pm 0.05$ and $r_2 = 0.18 \pm 0.01$, respectively. Water-soluble copolymers of varying compositions and molecular weights demonstrating high antiviral activity are synthesized.

**DOI:** 10.1134/S1560090423700793

Respiratory viral infections are still an important health issue despite significant achievements of vaccine therapy. Vaccine-resistant strains arise very rapidly. In addition, the transportation and storage of vaccines require strict adherence to a certain temperature regime. During seasonal peaks of respiratory viral infections the need for new effective antiviral chemotherapeutic agents is especially urgent. A solution of this issue implies search and development of new broad-spectrum antiviral agents with low toxicity. Among promising antiviral compounds macromolecular compounds are of particular interest. As early as the mid-20th century it was found that some water-soluble synthetic polymers possess the antiviral activity: anionic polyelectrolytes, poly(acrylic acid) and poly(methacrylic acid), maleic anhydride copolymers, polymeric derivatives of *p*-aminosalicylic acid, and polymers containing sulfo and phosphate groups [1–7]. High antiviral activity is also exhibited by natural polymers—sulfates and phosphates of polysaccharides [8, 9]. Their activity is due to their ability to block the interaction of viruses with a host cell, induce production of interferon in human body, activate macrophages, and enhance nonspecific body resistance to external infections. Amongst a large number of various polyanions polystyrene sulfonate, which is active against respiratory viruses, has been studied most extensively [9, 10]. It is especially active as an anti-HIV agent and has antiviral activity against a broad spectrum of sexually transmitted diseases. The modification of polystyrene sulfonate by introducing units of a different structure is of interest for gaining insight into effect of the chemical structure of copolymers on antiviral activity and searching for new polymers with the enlarged spectrum of action and a higher activity.

This study addresses the modification of polystyrene sulfonate by the copolymerization of sodium styrene sulfonate ($M_1$) with the neutral hydrophilic monomer 2-deoxy-2-methacrylamido-D-glucose ($M_2$), the synthesis of new copolymers of varying compositions and molecular weights, and the investigation of their antiviral activity and cytotoxicity.

In this work, sodium styrene sulfonate (**Na-SS**) purchased from Aldrich (Germany) was used. The initiator was 2.2-azobis(methylpropionamidine) dihydrochloride (**MPC**) (Aldrich, Germany).

The monomer 2-deoxy-2-methacrylamido-D-glucose (**MAG**) was obtained by the known Klein method [11] through the acylation of glucosamine hydrochloride by methacryloyl chloride in the presence of triethylamine. After recrystallization its $T_m$ was 197–198°C, in agreement with the published data.

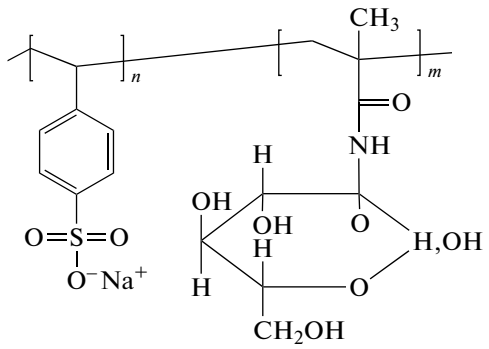
Copolymers Na-SS–MAG with varying molar ratios and molecular weights were synthesized by free-radical polymerization in water using water-soluble initiator MPC in an argon atmosphere in sealed ampoules at a temperature of 60°C for a day. The copolymers were purified from low molecular weight components by dialysis against water using a semipermeable membrane (Biolot) (molecular weight cutoff 1 kDa) and isolated by lyophilization. The conditions of copolymerization and the characteristics of the synthesized copolymers Na-SS–MAG are presented in Table 1.

**Table 1.** Yields and molecular weights of Na-SS copolymers (solvent, water; $[M_1] + [M_2] = 20$ wt %; MPC, 3 wt %; $T = 60°C$; time, 24 h)

| Content of MAG in the initial mixture, mol % | Yield, wt % | Content of $M_2$ units in copolymer, mol % | $[\eta]^{25}$ in 0.2 NaCl, dL/g | $M \times 10^{-3}$ |
|---|---|---|---|---|
| 10 | 61 | 23 | 1.45 | 850 |
| 20 | 66 | 29 | 1.25 | 690 |
| 30 | 68 | 34 | 1.0 | 490 |
| 50 | 88 | 51 | 1.10 | 570 |
| 0* | 90 | – | 0.63 | 255 |

* The homopolymer Na-PSS was synthesized by radical polymerization in DMF in the presence of 1 wt % AIBN.

The intrinsic viscosity was measured at 25°C in 0.2 M NaCl with an Ubbelohde viscometer. The molecular weight range of the copolymers was calculated by the Kuhn–Mark–Houwink equation for sodium polystyrene sulfonate (**Na-PSS**) in 0.2 M NaCl at 25°C: $[\eta] = 1.17 \times 10^{-2} \times M^{0.69 \pm 0.06}$ cm$^3$/g [12].




Na-SS–MAG

To estimate the relative activities of Na-SS and MAG copolymerization was carried out to a conversion of 10 wt % at the initial molar ratio of comonomers from 90 : 10 to 10 : 90. When calculating the relative reactivity of the comonomers the mathematical treatment of the experimental data by the Fineman–Ross method was used [13]. Figure 1 shows the composition of Na-SS–MAG copolymers versus the composition of the initial comonomer mixture. It was found that for Na-SS $r_1 = 1.58 \pm 0.05$ and for MAG $r_2 = 0.18 \pm 0.01$. The values of $r_1$ and $r_2$ indicate a higher reactivity of Na-SS. The resulting copolymers are enriched in Na-SS units.

The composition of the copolymers was determined by UV spectroscopy at a wavelength of 260 nm using a Shimadzu UV-1280 spectrophotometer. IR spectra were recorded on a Shimadzu IRAffinity-1S spectrometer using the KBr pellet technique (Fig. 2). The spectra of the copolymers Na-SS-MAG show bands typical of both types of units. The presence of Na-SS units was judged from the absorption band at ~1450–1580 cm$^{-1}$ corresponding to the benzene ring. In the spectra of the copolymers there are absorption bands corresponding to S=O units (1050–1200 cm$^{-1}$). Vibration bands of $CH_3$- and $CH_2$- groups at 2900 and 1550 cm$^{-1}$ correspond to MAG units. There are also

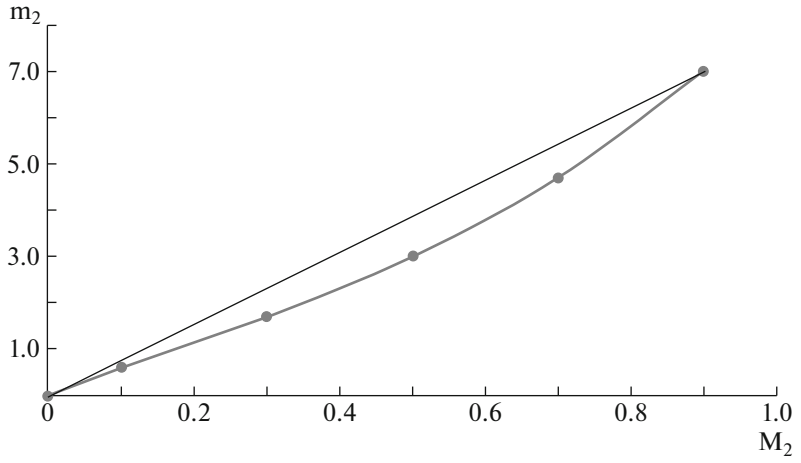



**Fig. 1.** Composition diagram for the copolymerization of Na-SS–MAG. $m_2$ is the molar fraction of MAG in the copolymer, and $M_2$ is the molar fraction of MAG in the monomer mixture.

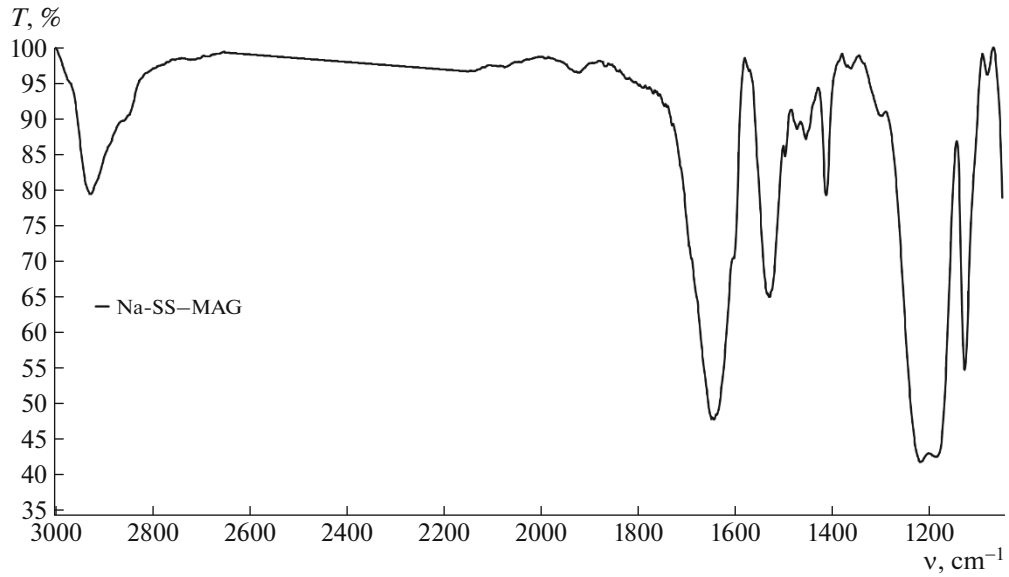



**Fig. 2.** IR spectrum of the copolymer Na-SS–MAG (49 : 51, mol %).

bands due to Amide I (1650 $cm^{-1}$) and Amide II (1524 $cm^{-1}$); the band at 1000 $cm^{-1}$ is due to vibrations of the pyranose ring.

The structure of the copolymers was additionally confirmed by $^1H$ NMR spectroscopy (400 MHz, $D_2O$). The $^1H$ NMR spectra of the copolymers exhibit signals due to protons of the glycosyl ring in MAG units ($\delta_H$ = 0.9–2.0, 3.3–3.8, 4.6–5.2 ppm) and a signal due to protons of the benzene ring $\delta_H$ = 7.4 ppm.

To determine cytotoxicity and antiviral activity the cell culture HEp-2, the epidermoid carcinoma of the human larynx, from the collection of cell cultures of the Smorodintsev Influenza Research Institute were taken. The human respiratory syncytial virus strain A2 obtained from the laboratory of biotechnology of diagnostic preparations was accumulated on the culture of cells HEp-2 and stored at a temperature of −80°C. The cytotoxicity index ($CTD_{50}$) of the polymers was estimated from cell viability by means of the microtetrazolium test [14]. The antiviral activity was determined by the enzyme-linked immunosorbent assay (ELISA). A series of three-fold polymer dilutions was prepared [15]. The criterion of antiviral activity was the statistically significant decrease in the virus titer in cells attained using the polymer compared with the control. On the basis of these data the value of $ED_{50}$ was calculated. To evaluate the prospects of using copolymers as antiviral agents, the value of chemotherapeutic index equal to the ratio of $CTD_{50}$ to $ED_{50}$ was used. Compounds having the chemotherapeutic index above eight were taken to be promising.

As is seen from Table 2, the Na-SS homopolymer and all the synthesized copolymers manifest a high antiviral activity and their cytotoxicity and activity depend on the copolymer composition and decrease with increasing content of vinyl saccharide units. The copolymers Na-SS–MAG (71–29 and 66–34 mol %) having the highest chemotherapeutic index compared with the homopolymer are of interest for further detailed research.

Thus, the radical copolymerization of sodium styrene sulfonate with methacrylamidoglucose in water was studied, the reactivity ratios of the comonomers were determined, and the copolymers of varying

**Table 2.** The cytotoxicity index ($CTD_{50}$) and the antiviral activity index ($ED_{50}$) of the synthesized copolymers Na-SS–MAG

| Content of MAG in the initial mixture, mol % | $M \times 10^{-3}$ | $CTD_{50}$, μg/mL | $ED_{50}$, μg/mL | Chemotherapeutic index |
|---|---|---|---|---|
| 23 | 850 | 37.3 | 1.0 | 39 |
| 29 | 690 | 35.6 | 0.3 | 137 |
| 34 | 490 | 90.5 | 1.1 | 82 |
| 51 | 570 | 110 | 5.7 | 19 |
| 0 | 255 | 163 | 3.5 | 47 |

compositions and molecular weights were synthesized. Their high antiviral activity against human respiratory syncytial virus was ascertained.

## ACKNOWLEDGMENTS

We are grateful to V.V. Glebenkova for her participation in the experimental work.

## FUNDING

This work was carried out within State Assignment: chemical part, the project registration number 122012100171-8, and biological experiments, the project registration number of RD in the Unified State RD Accounting Information System 121051900142-0 "Genetic and antigenic variability of modern strains of respiratory syncytial viruses (RSV) circulating on the territory of the Russian Federation."

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## REFERENCES

1. W. Regelson, Adv. Chemother. **3**, 303 (1968).
2. N. A. Zeitlenok, L. M. Vil'ner, L. B. Trukhmanov, V. A. Kropachev, I. M. Rodin, T. M. Markelov, and M. M. Gol'dfarb, Vopr. Immunol., No. 4, 401 (1968).
3. D. I. Ershov, I. A. Vinogradov, M. M. Kozlovskii, V. A. Pendarskii, and L. L. Stotskaya, in *Polymers for Medical Applications* (INKhS, Moscow, 1988) [in Russian].
4. T. S. Abdukhodzhaev, V. A. Kropachev, L. B. Trukhmanova, and L. M. Vil'ner, in *Physiologically Active Polymeric Compounds* (Izd. TashGU, Tashkent, 1976) [in Russian].
5. E. De Clercq, Collect. Czech. Chem. Commun. **63**, 450 (1998).
6. F. Schandck, C. F. Riber, and A. Rocker, Adv. Healthcare Mater. **6** (23), 1700748 (2017).
7. V. V. Zarubaev, E. V. Buchkov, O. V. Nazarova, Yu. I. Zolotova, and E. F. Panarin, Dokl. Biokhim. Biofiz. **506**, 227 (2022).
8. M. Jabeen, M. Dutot, R. Fagon, B. Verrier, and C. Monge, Pharmaceutics **13** (5), 733 (2021).
9. A. Anderson, K. Feathergill, X. Diao, M. Cooper, R. Kirkpatrick, P. Spear, D. Waller, C. Chany, G. Doncel, B. Herold, and L. Zaneveld, J. Andrology **21** (6), 862 (2000).
10. N. A. Kontarov, A. A. Ermakova, N. S. Grebenkina, N. V. Yuminova, and V. V. Zverev, Vopr. Virusol. **60** (4), 5 (2015).
11. J. Klein and D. Herzog, Makromol. Chem. **188** (6), 1217 (1987).
12. G. M. Pavlov, I. I. Zaitseva, A. S. Gubarev, I. I. Gavrilova, and E. F. Panarin, Russ. J. Appl. Chem. **79** (9), 1490 (2006).
13. M. Fineman and S. D. Ross, J. Polym. Sci. **5** (2), 259 (1950).
14. M. Niks and M. Otto, J. Immunol. Methods **130**, 149 (1990).
15. L. J. Reed and H. Muench, J. Am. Hygiene **27**, 493 (1983).

*Translated by T. Soboleva*