Pergamon

PII: S0014-3057(96)00036-5

Eur. Polym. J. Vol. 32, No. 8, pp. 957–962, 1996

0014-3057/96 $15.00 + 0.00

OLIGOAMIDOAMINES AND OLIGOESTERAMINES BASED ON ANTIBIOTICS CONTAINING β-LACTAM RING

DO THI BICH LOAN** and IVAN M. PANAYOTOV*

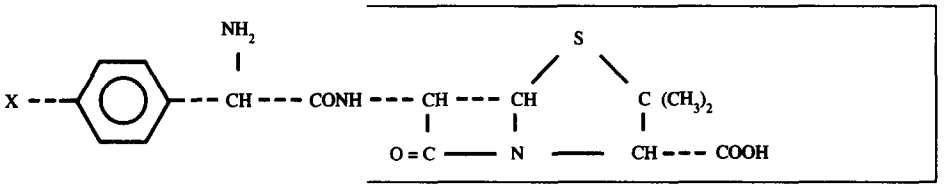
Institute of Polymers, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

(Received 28 June 1995; accepted in final form 9 October 1995)

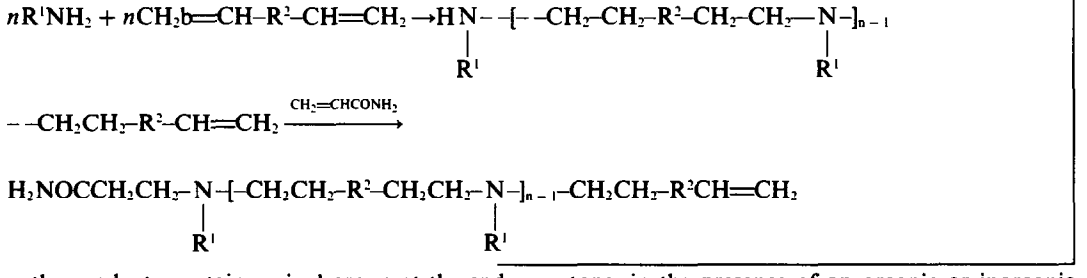
Abstract—The preparation of polyamidoamines and polyesteramines by interaction between ampicillin and amoxicillin and methylene-bis-acrylamide, 1,4-diacryloylpiperazine and 1,3-propanedioldiacrylate is described. The prepared polymers contain a vinyl group at the end of the macromolecules which can be copolymerized with other monomers. All polymer products exhibit anomalous behaviour in solution, typical of polyelectrolytes. Copyright © 1996 Published by Elsevier Science Ltd

INTRODUCTION

In our previous work [1, 2] we described the preparation and properties of polyamidoamines (PAA) and polyesteramines (PEA) from physiologically active aromatic and aliphatic aminoacids. The aim of this paper is to prepare the same type of products from two largely used antibiotics containing β-lactam rings: ampicillin (X = H) and amoxicillin (X = OH)

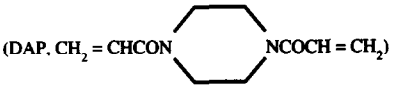


As is known, PAA and PEA are prepared via polyaddition of amines and amides or esters containing two activated double bonds [3–8]. The polymers obtained are stabilized by reacting with a monofunctional unsaturated amide.



Thus, the products contain a vinyl group at the end of the macromolecules, i.e. they can be regarded as macromonomers.

The compounds with activated double bonds used in this work were: methylene-bis-acrylamide (MBAA, CH_2=CHCONH–CH_2–NHOCCH=CH_2), 1,4-diacryloylpiperazine



and 1,3-propanedioldiacrylate (PDDA, CH_2=CHCOO(CH_2)$_3$OOCCH=CH_2). According to literature data [9], the reactions with the amine group of the two antibiotics are performed mostly in a mixture of water and organic solvent, usually acetone, in the presence of an organic or inorganic base (sodium bicarbonate or triethylamine). There are some difficulties in the isolation of the reaction products due to the similar solubility of the reagents. The chemical sensitivity of the β-lactam ring should also be considered.

EXPERIMENTAL METHODS

The antibiotics were received as gifts with pharmacological purity as trihyrates from the firm Antibiotics, Razgrad,

*To whom all correspondence should be addressed.
**Permanent address: Centre for Polymers, National Centre of Scientific Research of Vietnam, Nghia Do-Tu Liem, Hanoi, Vietnam.

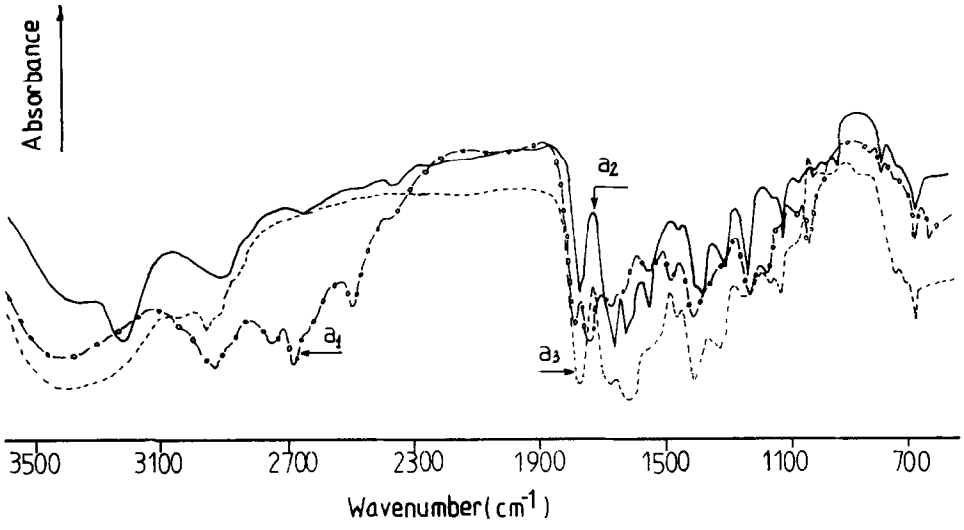


Fig. 1. IR spectra of the products prepared in the interaction between MBAA and ampicillin. a_1, free acid of the product no. 1 from Table 1; a_2, triethylammonium salt of the product no 2 from Table 1, a_3, sodium salt of the product no. 3 from Table 1

Bulgaria and used without further purification The other reagents were of the same origin and purity as our previous communications [1, 2].

General procedure

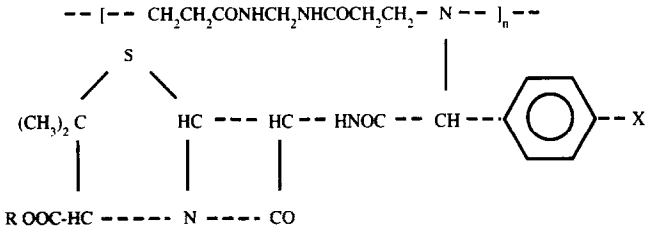
0.01 mol of ampicillin–trihydrate or amoxicillin–trihydrate (4.04 and 4.20 g, respectively) were dissolved in 10 ml of water and 30 ml of acetone. The mixture was cooled to 0–5 C. 0.01 mol (1.44 ml) of triethylamine was added to the mixture and stirred to complete dissolution 0 01 mol of the product with activated double bonds was then added to the mixture, stirred for other 2 hr at 0–5 C and left at ambient temperature for 3–4 days The solvent was removed *in vacuo*. The residue was dissolved in methanol and poured into excess diethyl ether upon stirring. The precipitate was reprecipitated several times from methanol solution in diethyl ether. Thus the triethylammonium salt of the polymer was prepared.

To obtain the free acid, the ammonium salt was dissolved in 10 ml of water and 30 ml of acetone. The mixture was cooled to 0–5°C and brought to pH about 2 with 4 N hydrochloric acid. The clear solution obtained was poured into excess diethyl ether upon stirring. The precipitate was reprecipitated several times from methanol solution in ether

Sodium salt of the polymer acid was prepared as follows. 0 01 g of amonium salt was dissolved in a small amount of methylene chloride. Separately, 0.12 g of sodium ethylcapronate was dissolved in 5 ml of methanol. The solution of sodium ethylcapronate was added dropwise upon stirring to the solution of ammonium salt, cooled to 0–5°C. The clear solution obtained was poured into excess isopropanol–*n*-hexane mixture (1 4) upon stirring. The precipitate was dried *in vacuo*, dissolved in dimethylformamide and reprecipitated several times in diethyl ether. The purity of the products obtained was checked by thin layer chromatography on Silicagel 60 and mobile phase isopropanol–water (7·3) in UV light

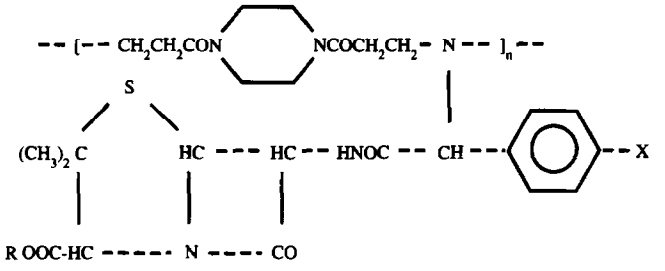
Copolymerization was performed with hydroxyethylmethacrylate (HEMA) according to the following procedure· 0.76 g of PAA, obtained from 1,4-DAP and ampicillin (product no. 2, Table 2), and 0.052 g of HEMA were dissolved in 20 ml of methanol and 0.002 g of benzoylperoxide was added followed by 0.001 g of dimethylaniline, both in methanol solution. The solution was kept for 3 days at room temperature, the solvent was

Table 1 Oligoamidoamines prepared from methylene-bis-acrylamide



No.	X	R	Yield (%)	m p (C)	*n* From ^{1}H NMR spectra
1	H	H	27 9	142–145	—
2	H	NEt_3	93 0	98–99	6
3	H	Na	39 1	250	—
4	OH	H	55 6	163–165	—
5	OH	NEt_3	69 5	104–105	3
6	OH	Na	38 9	> 260	—

Table 2. Oligoamidoamines prepared from 1,4-diacryloylpiperazine



No.	X	R	Yield (%)	m.p (°C)	*n* From ^{1}H NMR spectra
1	H	H	45.0	217–220	—
2	H	NEt_3	90.0	80–85	3
3	H	Na	54.0	251	—
4	OH	H	48.6	135–138	—
5	OH	NEt_3	90.0	95–100	6
6	OH	Na	57.6	> 260	—

distilled *in vacuo* and the product obtained was dissolved in methanol and precipitated in acetone under stirring. The precipitate was washed several times with water to remove the unreacted monomers and then dissolved in methanol and reprecipitated in diethyl ether. Yield 0.48 g, m.p. 218–222°C.

The viscosity of the polymer solutions was determined in water or phosphate buffers on a capillary viscometer Ubbelohde type. IR spectra were recorded on a Specord M80 apparatus. A Brucker apparatus was used to record ^{1}H NMR spectra at 250 MHz and 297 K in deuterated DMSO-d_6 with tetramethylsilane as internal standard.

RESULTS AND DISCUSSION

The prepared polymers are highly hygroscopic, colourless substances. They are well soluble in water (with the exception of the products with a free carboxyl group), in dilute mineral acids, methanol, dimethylformamide and dimethylsulfoxide. They are not soluble in ether, aliphatic and aromatic hydrocarbons.

IR spectra. All characteristic peaks of the functional groups, including those of the β-lactam ring (1750–1780 cm^{-1}), are found in the IR spectra. As an example, Fig. 1 shows the IR spectra of products 1–3 from Table 1.

^{1}H NMR spectra. In the ^{1}H NMR spectra no peaks for the free amino group were registered, but all signals characteristic for protons of the structure were found in the spectra.

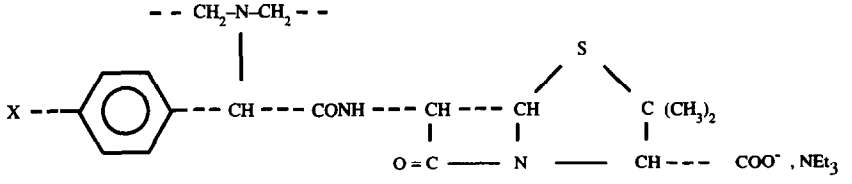
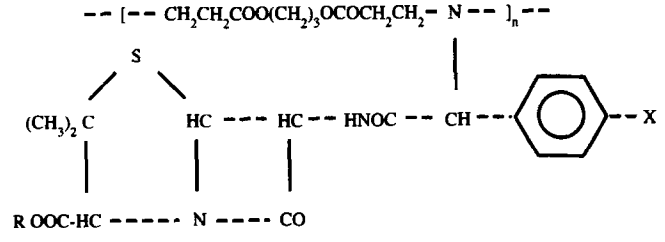


Table 3. Oligoesteramines prepared from 1,3-propanedioldiacrylate



No	X	R	Yield (%)	m.p (°C)	*n* from ^{1}H NMR spectra
1	H	H	17.4	168–170	—
2	H	NEt_3	54.5	105–110	no signals for unsaturation
3	H	Na	27.3	248–250	—
4	OH	H	17.9	193–195	—
5	OH	NEt_3	66 3	110–115	10
6	OH	Na	59.7	255–256	—

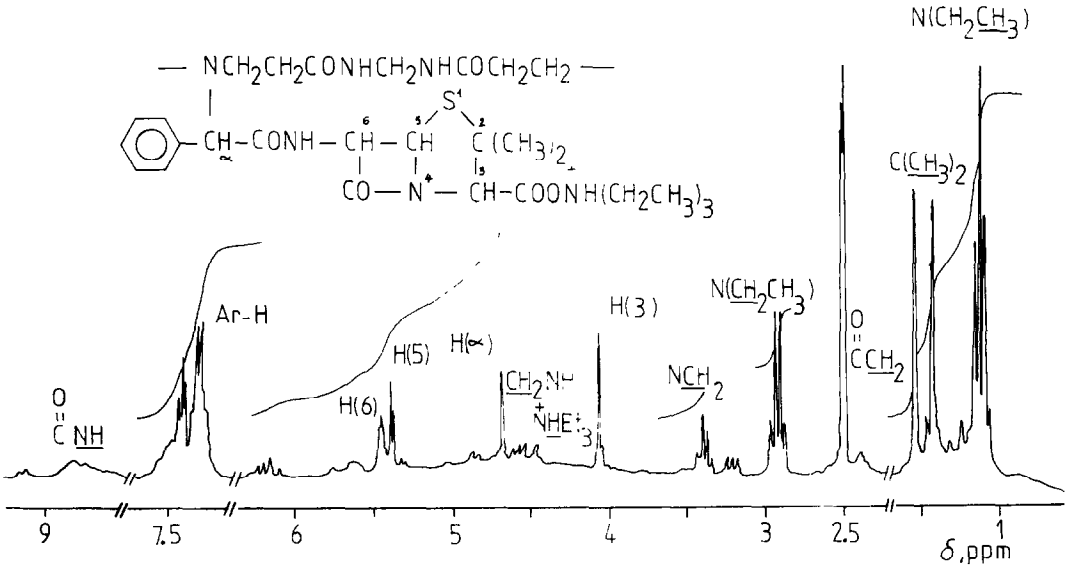
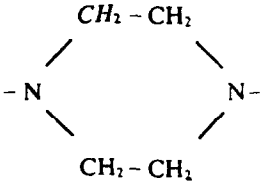


Fig. 2. ^{1}H NMR spectrum of the polymer prepared from MBAA and ampicillin (product no. 2 from Table 1)



$COCH_2$ – 4H, triplet at 2.42 ppm

$CONH$ – 2H, triplet at 8.75 ppm

$N–CH_2$ – 2H, multiplet with centre at 3.53 ppm

$COOCH_2$ – 2H, triplet at 4.18 ppm

$COOCH_2CH_2$ – 2H, triplet at 2.63 ppm

$NHCH_2NH$ – 2H, triplet at 4.47 ppm

NCH_2CH_3 – 6H, quadruplet at 2.88–2.96 ppm

NCH_2CH_3 – 9H, triplet at 1.18 ppm

8H, multiplet with centre at 3.51 ppm

$(CH_3)_2$ – 6H, singlet at 1.47–1.53 ppm

H^7 – 1H, singlet at 4.67 ppm

H^5, H^6 – 2H, two doublets at 5.37–5.41 ppm

H^3 – 1H, singlet at 4.06 ppm

aromatic ring (5H, at 7.20–7.35 ppm).

As an example, Fig. 2 shows ^{1}H NMR spectra of the product prepared from MBAA and ampicillin (product no. 2, Table 1).

Elemental analyses

In most cases the elemental analyses of oligomers did not show good reproducibility (differences up to 1 6% in the nitrogen content), although in some cases the differences were between 0.20 and 0.50%. Presumably, the hygroscopicity of the oligomers plays an important role.

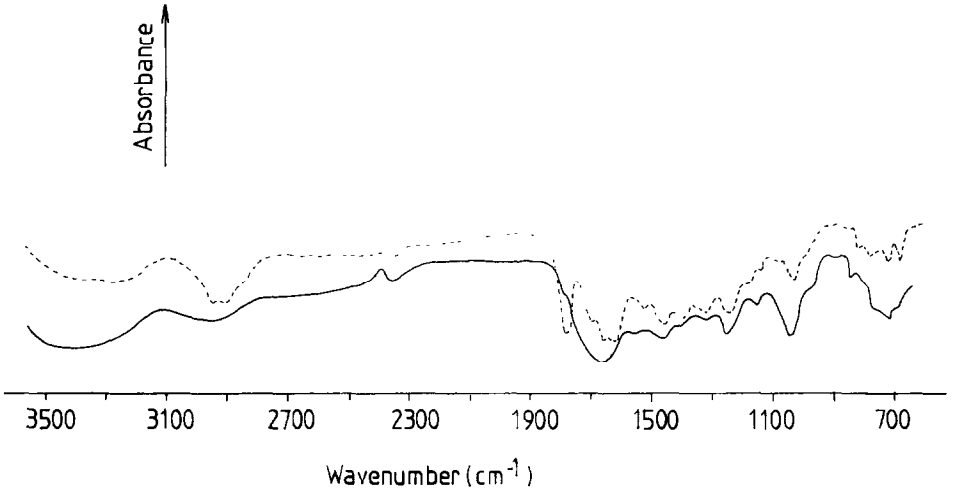


Fig. 3. IR spectra of copolymer (—) and monomer PAA (- - - -).

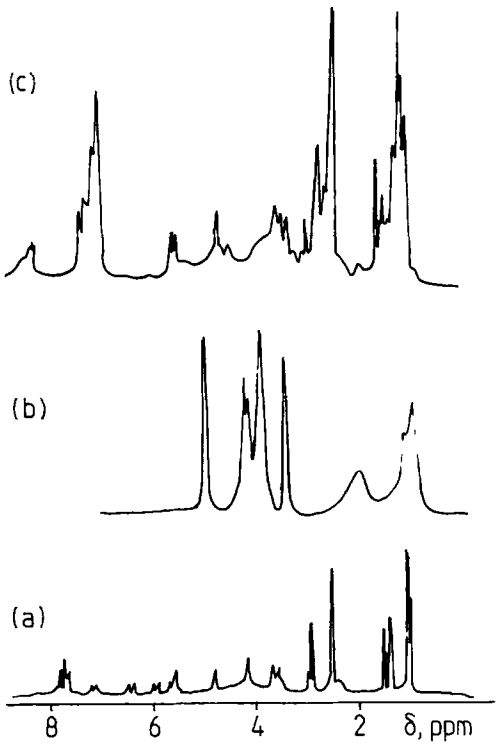


Fig. 4. ^{1}H NMR spectra of polymers: (a) PAA in DMSO; (b) poly(HEMA) in CD_3OD; (c) copolymer in DMSO.

Molecular weights

No reliable data for molecular weight could be obtained from gel permeation chromatography. The results show that associates (micelles) are formed in the solution. Some information can be obtained from the unsaturation determined by ^{1}H NMR spectra (peaks at 5.50–6.87 ppm, see tables). The results indicate that in most cases the products obtained are mainly oligomers.

The copolymer of PAA and HEMA is a colourless solid, soluble in methanol, DMSO and dilute mineral acids, insoluble in water, acetone, diethyl ether and hydrocarbons. Its structure was established by IR and ^{1}H NMR spectra. The IR spectrum (Fig. 3) indicates all characteristic peaks for the functional groups for PAA, including for the β-lactam ring (1750–1780 cm^{-1}), as well as for the functional groups of HEMA: at 1720, 1150 and 1065 cm^{-1} for $\nu_{C=O}$, ν_{C-O} (ester) and ν_{C-O} (alcohol). Figure 4 represents the ^{1}H NMR spectrum of the copolymer and of the starting monomers. The signals at 3.90 and 4.20 ppm are characteristic of the methylene groups of poly(HEMA). The signals at 3.51 ppm (piperazine ring) and at 7.26–7.63 ppm (aromatic ring) are characteristic of the starting PAA. These results show that the resulting polymer possesses both poly (HEMA) and PAA portions.

The ability of the obtained polymers to copolymerize reveals interesting possibilities for preparation of different graft copolymers, including polymers with complex biological activity.

Dilute solution viscosity

The dependence of reduced viscosity on concentration is not linear. Its value decreases with the concentration, which is typical of polyectrolytes. Only in individual cases when using the Fuoss–Strauss equation [10]:

$$1/\eta_{red} = 1/[\eta] = BC^{0.5}$$

was it possible to show that the reciprocal value of the reduced viscosity depends on the square root of the

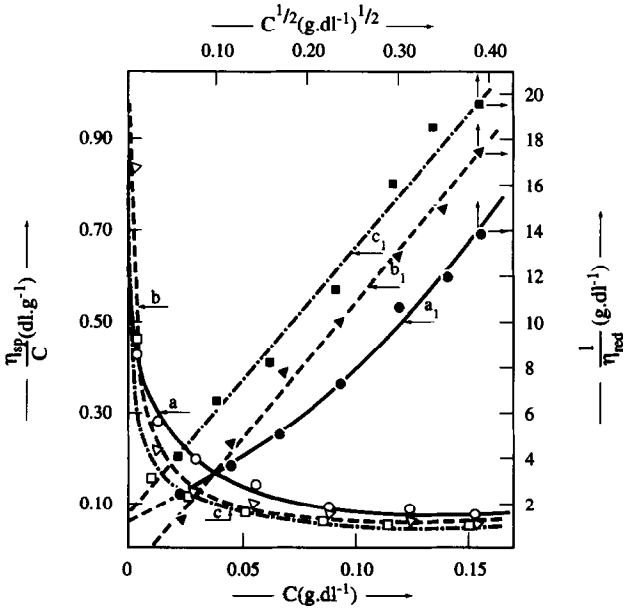


Fig. 5. Dependence of the reduced viscosity on the concentration of the polymer solutions: a, a_1, product no. 2 from Table 1, phosphate buffer, pH 7.2, 25°C; b, b_1, product no. 2 from Table 2, phosphate buffer, pH 7.2, 25°C; c, c_1, product no. 5 from Table 3, distilled water, 30°C.

concentration, which allows estimation of the intrinsic viscosity. In most cases the Fuoss equation led to inexplicable results. Figure 5 shows some dependences of the viscosity on the concentration of the obtained polymers. Since the β-lactam ring is very sensitive, it was impossible to follow the dependences in a stronger acid or stronger alkaline medium.

Future work

Investigations on the behaviour of polyamidoamines and polyesteramines in solution, including the determination of molecular weights, as well as on biological activities, are in progress and will be published later.

REFERENCES

1. Do Thi Bich Loan, I. B. Rashkov and I. M. Panayotov *C. R. Acad. Bulg. Sci.* (in press).
2. Do Thi Bich Loan, I. B. Rashkov and I. M. Panayotov. *Macromol. Chem. Phys.* **196,** 3525 (1995).
3. F Danusso and P. Ferruti. *Polymer* **11,** 88 (1970).
4. P Ferruti and R. Barbucci. *Adv. Polym. Sci* **58,** 55 (1984).
5. P Ferruti, M. A. Marchisio and R Barbucci. *Polymer* **26,** 1336 (1985).
6. R. Barbucci, M. Casdaro, M. Nocentini, Scorezzi, P. Ferruti and V Barone. *Macromolecules* **19,** 37 (1986).
7. P. Ferruti and M. A. Marchisio. In *Polymer Science and Technology* (edited by Charles G. Gebelein and Frank F Koblitz), Vol. 14, p. 39. Plenum Press, New York (1981)
8. A N. Shtopenko, V. A. Shulga, L V. Shtyrlina, O. V. Kargina and V. A. Kabanov. *Vysokomol. soed* **A32,** 2135 (1990).
9. A Nakov *et al.* Bulg. Pat. 24865 (1977); 268869 (1977)
10. C Tanford. *Physical Chemistry of Macromolecules*, p 560. Chimia, Moscow (1965) (Russian translation).