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MACROMOLECULAR CHEMISTRY AND POLYMERIC MATERIALS

Synthesis of Low-Molecular-Weight Copolymers of *N*-Vinylpyrrolidone with 2-Hydroxyethyl Methacrylate and of Polymeric Oxacillin Esters Derived from Them

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Abstract—The radical copolymerization of *N*-vinylpyrrolidone with 2-hydroxyethyl methacrylate in 2-propanol at 60°C, initiated by azobis(isobutyronitrile) and inhibited by mercaptoethanol or terminated after a short period, was studied. The low-molecular-weight copolymers obtained were used to prepare new polymeric forms of Oxacillin antibiotic. The chemical uniformity, composition, and antimicrobial activity of the new derivatives were studied, and the rate of their hydrolysis in saline (pH 7.3) and glycine buffer (pH 2.0) was determined.

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In the development of polymeric carriers based on *N*-vinylpyrrolidone (VP) for biologically active substances (BASs) [1], much promise is shown by copolymers of VP with 2-hydroxyethyl methacrylate (HEMA), which are hydrophilic, nontoxic, and biocompatible with a living body.

Previously [2] we studied the copolymerization of VP (M_1) with HEMA (M_2) and determined the compositions and molecular weights of the resulting copolymers. The copolymerization constants obtained, $r_1 = 0.29 \pm 0.02$ and $r_2 = 6.68 \pm 0.34$, showed that HEMA is considerably more active comonomer than VP. As a result, the calculated running composition of the copolymer is enriched in HEMA units at any composition of the starting monomer mixture (Fig. 1). This results in formation of copolymers of nonuniform composition. More compositionally uniform copolymers are formed only in copolymerization of monomer mixtures enriched in VP. The molecular weight (MW) of the resulting VP–HEMA copolymers, 50 000–90 000 Da, exceeded the limiting MW of synthetic non-biodegradable polymers (40 000 Da) [3] passing through kidneys.

The goal of this study was to prepare low-molecular-weight (MW = 20 000–30 000 Da) VP–HEMA copolymers and to examine the possibility of their use as new polymeric BAS carriers.

To obtain low-molecular-weight copolymers, we performed the copolymerization of VP with HEMA in 2-propanol, initiated by azobis(isobutyronitrile) (AIBN), in the presence of a chain-terminating agent, mercaptoethanol, added in an amount of 0.1–0.2 wt % relative to the sum of the comonomers, or terminated the copolymerization after a short period. The conditions of the synthesis of low-molecular-weight VP–

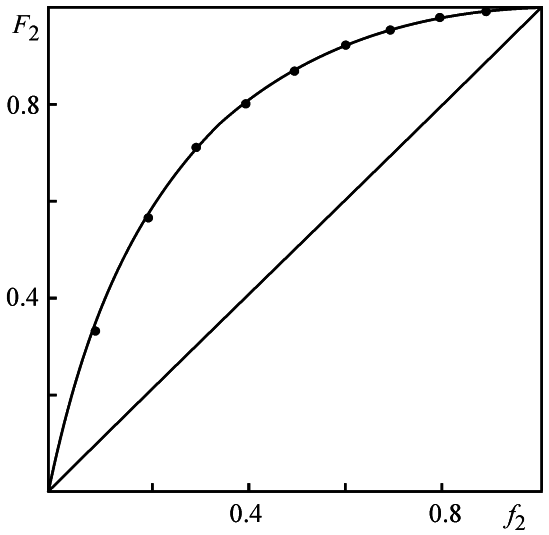
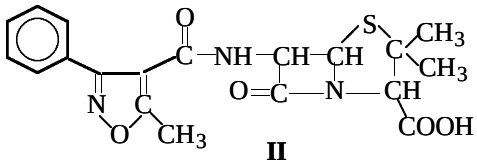


Fig. 1. Running composition of VP (M_1)–HEMA (M_2) copolymers as a function of the composition of the starting monomer mixture: (f_2) mole fraction of HEMA in the starting mixture and (F_2) mole fraction of HEMA units in the VP–HEMA copolymer.

HEMA copolymers **I** and their characteristics (compositions, diffusion coefficients D, sedimentation coefficients S_0, intrinsic viscosities, molecular weights M_{SD}) are given in Table 1. It is seen that, under these conditions, low-molecular-weight (M_{SD} = 16000–31000 Da) VP–HEMA copolymers are obtained in 53–65% yield. With an increase in the mercaptoethanol content in the starting mixture, the yield and MW of the resulting copolymer decrease, which is apparently associated with an increase in the chain termination rate. Copolymers **Ia**–**Ic** are enriched in HEMA units relative to the composition of the starting monomer mixture. This effect becomes more pronounced with a decrease in the polymer yield. Copolymers **Ib** and **Ic** are readily soluble in water and contain a large amount (25.8–29.4 mol %) of hydroxyl-containing HEMA units, which allows their use for modifying Oxacillin antibiotic.

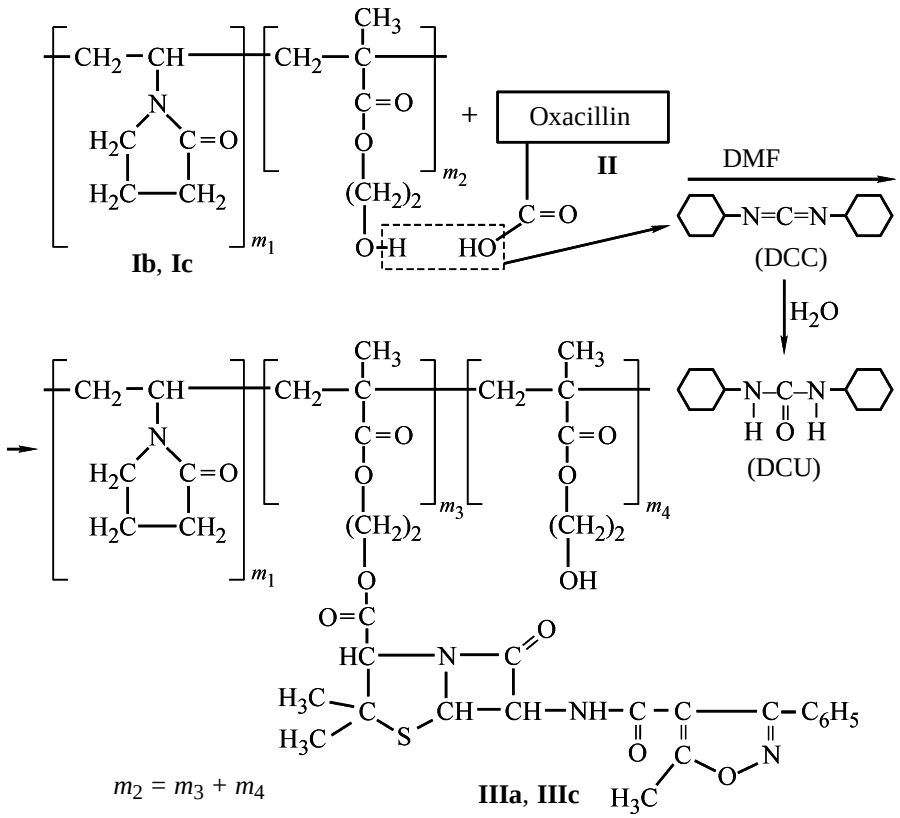
Oxacillin **II** is a semisynthetic penicillin [4] which is effective against penicillinase-forming staphylococci, acid-resistant, and nontoxic; it is widely used in clinical practice in the form of sodium salt:



II

However, the major drawback of Oxacillin is its short therapeutic effect. To maintain the therapeutic concentration of Oxacillin in blood, it should be administered every 4 h. To develop polymeric forms of Oxacillin of prolonged effect, the antibiotic was modified with copolymers **Ib** and **Ic**.

Condensation of Oxacillin with VP–HEMA copolymers **Ib** and **Ic** was performed in DMF at 0–5°C in the presence of a condensing agent, *N*,*N*'-dicyclohexylcarbodiimide (DCC). In so doing, the antibiotic was linked to the polymeric carrier by an ester bond:



The evidence of the reaction was formation of an abundant precipitate of the by-product, dicyclohexylurea (DCU).

By gel filtration (Sephadex G-50 [5]) we confirmed the chemical uniformity of condensation products **IIIa** and **IIIb** and the absence of free Oxacillin impurities.

Table 1. Copolymerization of VP with HEMA in 2-propanol at 60°C and properties of copolymers **I** ([AIBN] = 4.5 wt %, $[M_1^0] : [M_2^0]$ molar ratio 80 : 20)

Starting monomer mixture, wt %		Copolymerization time, h	Copolymer	Yield, %	*N*, %	m_2, mol %	[η], dl g^{-1}, 25°C, DMF	$D \times 10^7$, cm^2 s^{-1}	$S_0 \times 10^{13}$, Sv	M_{SD}, Da
$[M_1^0 + M_2^0]$	mercaptoethanol									
7.5	0.2	24	**Ia**	53.4	7.95	33.4	0.07	6.6	1.0	16 000
7.5	0.1	24	**Ib**	63.2	8.48	29.4	0.11	4.6	1.2	27 000
10.0	–	2	**Ic**	65.0	8.92	25.8	0.13	7.1	1.9	31 000

The structure of Oxacillin polyesters **IIIa** and **IIIb** was confirmed by UV and IR spectroscopy and also by quantitative iodometric analysis for penicillins [6]. As seen from Figs. 2 and 3, in the UV spectra of aqueous solutions of Oxacillin sodium salt and polymeric Oxacillin ester **IIIa** there is a shoulder at 230–250 nm, absent in the spectrum of an aqueous solution of the starting polymer **Ic**. This fact confirms the presence of Oxacillin in **IIIa** and allows estimation of the Oxacillin content in the product. Figure 4 shows the IR spectra of VP–HEMA copolymer **Ic** and polymeric Oxacillin ester **IIIa** derived from it. It is seen that a new strong band at about 750 cm^{-1}, absent in the spectrum of **Ic** and belonging to bending vibrations of the –CH= groups of the benzene ring in the Oxacillin molecule, appears in the spectrum of **IIIa**. The stretching vibration band of the ester C=O groups at about 1720 cm^{-1} in the spectrum of **IIIa** is stronger than in the spectrum of **Ic** owing to addition of Oxacillin to **Ic** through an ester bond. Finally, the methyl stretching vibration band at about 2900 cm^{-1} in the IR spectrum of **IIIa** is broadened relative to the corresponding band in the spectrum of **Ic**. This fact is also indicative of the incorporation of Oxacillin containing three CH_3 groups into the polymeric product.

The compositions of polymeric Oxacillin esters **IIIa** and **IIIb** are given in Table 2. It is seen that the Oxacillin content determined spectrophotometrically and iodometrically coincides within the analytical error. This means that Oxacillin is not inactivated in the course of the DCC-induced esterification (the β-lactam ring, actually determined in the iodometric procedure, is preserved).

The content of the incorporated Oxacillin in polyesters **IIIa** and **IIIb** is high (30–33%). The degree of conversion *Q* of the hydroxy groups of the polymeric carriers in their reaction with Oxacillin, calculated from these data, is 64–66%. This high value of *Q* indicates that the above-noted microstructural features of the starting VP–HEMA copolymers do not interfere with their reaction with Oxacillin molecules.

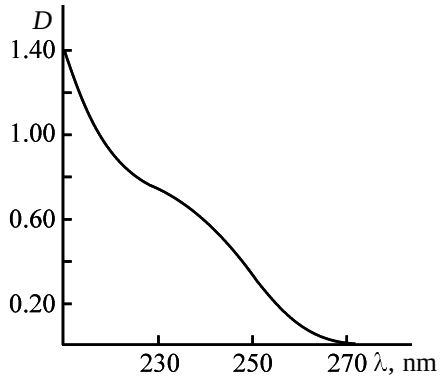


Fig. 2. UV spectrum of an aqueous solution of Oxacillin sodium salt (c = 25.7 μg ml^{-1}): (*D*) optical density and (λ) wavelength; the same for Fig. 3.

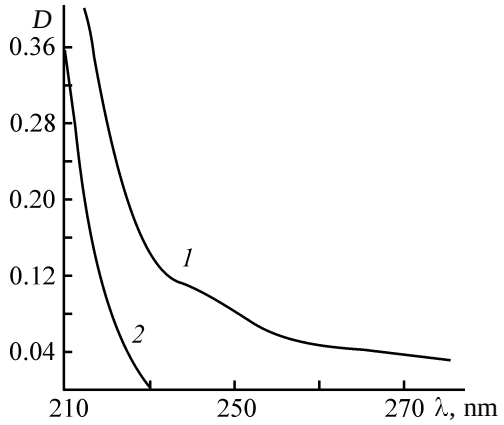


Fig. 3. UV spectra of aqueous solutions of (*1*) polymeric Oxacillin ester **IIIa** (c = 10.9 μg ml^{-1}) and (*2*) starting copolymer **Ic** (c = 19.7 μg ml^{-1}).

We found that polymeric Oxacillin ester **IIIa** exhibits high antimicrobial activity *in vitro*. When taken in concentrations of 3×10^{-3}–3×10^{-4} μg ml^{-1}, it killed standard staphylococcus strains (Table 2).[1]

[1] The authors are grateful to T.S. Potekhina for the determination of the antistaphylococcus activity of Oxacillin sodium salt and polymeric Oxacillin ester **IIIa**.

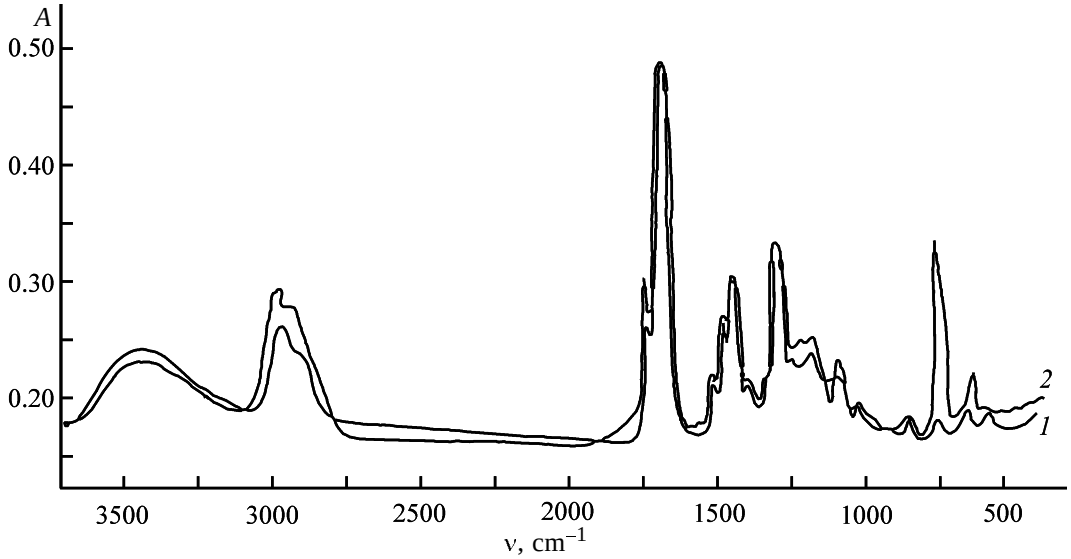


Fig. 4. IR spectra (films prepared from chloroform solution): (*A*) absorption and (ν) wavenumber. (*1*) Copolymer **Ic** and (*2*) polymeric Oxacillin ester **IIIa**.

As Oxacillin is administered parenterally and perorally, it seemed appropriate to estimate by dialysis through a semipermeable cellophane membrane the rate of cleavage of the ester bond between the antibiotic and polymeric carrier in the following model media: (a) saline, pH 7.3, 37°C; (b) glycine buffer solution, pH 2.0, 37°C. We found (Table 3) that the hydrolysis of **IIIa** in both media occurs at a noticeable rate even in the first hours of the reaction. As expected, at pH 2.0 the reaction is faster: 58% of the antibiotic is eliminated in 7 h and 84%, in 24 h. In saline, the degree of hydrolysis of **IIIa** is 44% in 7 h and 69% in 24 h.

The relatively high rate of hydrolysis of **IIIa** at both pH 2.0 and pH 7.3, in combination with the high antimicrobial activity of the preparation, indicate that the chosen approach to development of Oxacillin preparations of prolonged effect for both peroral and parenteral administration shows much promise.

EXPERIMENTAL

N-Vinylpyrrolidone was purified by threefold vacuum distillation from granulated KOH (polymerization inhibitor) in the presence of KU-2 cation-exchange resin sorbing amine impurities present in VP. The fraction boiling at 75–76°C/5–6 mm Hg was collected. Found: n_D^{20} = 1.5110, d_4^{20} = 1.0430 g cm^{-3}. Published data: n_D^{20} = 1.5130, d_4^{20} = 1.0458 g cm^{-3}. Found: molecular refraction R = 31.88 cm^3 mol^{-1}; calculated: R = 31.65 cm^3 mol^{-1}.

2-Hydroxyethyl methacrylate (Fluka) was distilled twice in a vacuum from molecular sieves in the presence of bis(naphthoquinonyl)-*p*-phenylenediamine (polymerization inhibitor). The fraction boiling at 87°C/5 mm Hg was collected. Found: n_D^{25} = 1.4500, d_4^{25} = 1.069 g cm^{-3}. Published data: n_D^{25} = 1.4505,

Table 2. Compositions and antistaphylococcus activity of polymeric Oxacillin esters

Copolymeric carrier	m_2, mol %	Ester	Yield, %	m_3	m_4	Oxacillin content, wt %		MBcC,* μg ml^{-1}	
				mol %		UV spectroscopy	iodometric titration	*St. aureus* 209 P	*St. aureus* 6538 P
Ic	25.8	**IIIa**	39.8	17.0	8.8	31.7	30.1	0.0003	0.003
Ib	29.4	**IIIb**	43.4	18.9	10.5	35.4	32.8	Not determined	
Oxacillin sodium salt (control)							92.5	0.0001	0.001

* (MBcC) Minimal bactericidal concentration recalculated to the Oxacillin content in the preparation.

d_4^{25} = 1.077 g cm^{-3}. Found: R = 32.68 cm^3 mol^{-1}; calculated: R = 32.62.

The initiator, azobis(isobutyronitrile) (AIBN), was recrystallized three times from chloroform–ethanol, 1 : 5. Yield of purified AIBN 76.2%, mp 103°C. Published data: mp 103°C.

Dimethylformamide was purified by the standard procedure [7]. Absolute 2-propanol, DCC (Fluka), and Oxacillin sodium salt (iodometric activity 925 μg mg^{-1}) were used without additional purification.

VP–HEMA copolymer Ib. An 80-ml glass ampule treated with live steam and dried was charged through a funnel with 0.121 g of AIBN and then with a mixture of 2 ml of VP, 0.58 ml of HEMA, 4 μl of mercaptoethanol, and 42 ml of 2-propanol. The ampule was purged with argon, sealed, and placed in a thermostat kept at 60°C. After heating for 24 h, the ampule was removed from the thermostat, cooled, and opened. The viscous transparent polymerization product was poured dropwise with stirring into a beaker containing 300 ml of diethyl ether. The precipitated copolymer was collected on a glass frit, washed with fresh portions of ether, and dried in a vacuum without heating to constant weight. Yield 1.71 g (63.2%). Found: N 8.48%.

Synthesis of Oxacillin from its sodium salt. Oxacillin sodium salt (2.5 g) was dissolved in 30 ml of water. The solution was transferred into a 250-ml separating funnel, and 50 ml of chloroform was added. To the aqueous layer, 1 N HCl was added dropwise, and the contents were shaken until the white precipitate in the aqueous layer disappeared. After the precipitation in the aqueous layer was complete, the acidification was stopped. The chloroform layer was separated, washed with three 30-ml portions of water, and dried over anhydrous sodium sulfate for 1 day at room temperature. The chloroform layer was separated, the solvent was removed on a rotary evaporator, and the residue was dried in a vacuum. Yield 1.78 g (78.4%). Iodometric activity 920 μg mg^{-1}. Found: S 7.84%; calculated: S 7.98%.

Polymeric Oxacillin ester IIIa. VP–HEMA copolymer **Ic** (0.5 g) was dissolved in 5 ml of DMF, and 0.445 g of dry Oxacillin was added. The mixture was stirred at cooling with an ice–salt mixture for 30 min, and a solution of 0.3 g of DCC in 3 ml of DMF was added. The solution was stirred for 2 h with cooling (0–5°C) and then for 5 h at room temperature. The mixture was left overnight in a refrigerator, after which the precipitate of dicyclohexylurea was filtered off, and the solution was poured into acetone to precipitate the reaction product (in so doing, unchanged Oxacillin, which is readily soluble in acetone, remained in the solution). After drying, 0.37 g (39.8%) of the water-soluble polymeric Oxacillin ester was obtained. The bound Oxacillin content was 31.7 wt % according to UV data and 30.1 wt % according to iodometric titration.

Table 3. Hydrolysis of polymeric Oxacillin ester **IIIa** at 37°C

Time from start of reaction, h	Degree of hydrolysis, %	
	pH 7.3	pH 2.0
1	12	27
2	21	36
3	29	44
4	34	47
5	37	50
6	40	54
7	44	58
24	69	84

The UV spectra of aqueous solutions of Oxacillin sodium salt, polymeric Oxacillin esters **IIIa** and **IIIb**, and copolymer **Ic** were recorded on a Specord M-40 spectrophotometer. The IR spectra of copolymer **Ic** and polymeric Oxacillin ester **IIIa** were measured on a Bruker IFS spectrometer; samples were prepared as films from chloroform solutions.

The molecular weights of copolymers **Ia**–**Ic** were determined by a sedimentation–diffusion procedure described elsewhere [2].

Hydrolysis of polymeric Oxacillin ester **IIIa** was studied in glycine buffer (pH 2.0) and saline (pH 7.3) at 37°C using the procedure described in [8].

The iodometric activity of Oxacillin and its polymeric esters was determined according to [6].

The antimicrobial activity of Oxacillin sodium salt and polymeric Oxacillin ester **IIIa** was studied by the method of tenfold serial dilutions in a liquid culture medium (meat–peptone broth, pH 7.0) [4]. The bacteria were grown for 18 h. The microbial load was 5×10^5 microbial bodies per milliliter.

CONCLUSIONS

(1) Conditions were found for preparing low-molecular-weight water-soluble *N*-vinylpyrrolidone–

2-hydroxyethyl methacrylate copolymers with a high (up to 33.4 mol %) content of 2-hydroxyethyl methacrylate units.

(2) From these copolymers, polymeric Oxacillin esters containing 30–33 wt % incorporated antibiotic and showing high antistaphylococcus activity *in vitro* were prepared.

(3) The hydrolysis of the polymeric Oxacillin ester with a molecular weight of 31000 Da at 37°C in model systems (glycine buffer, pH 2.0; saline, pH 7.3) was studied. In both systems, the hydrolysis of the polymeric ester is fairly fast: The degree of hydrolysis in 24 h is 84% in glycine buffer and 69% in saline.

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