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Structure vs release-rate relationships in polymeric esters of plant growth regulators

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Polymeric derivatives of plant growth regulators (phytoactive polymers) demonstrate their biological activity after releasing the low-molecular-weight bioactive substances by hydrolysis. In the present work the release rates for a series of polymeric esters of carboxyl-containing plant growth regulators of the auxin group (2,4-dichlorophenoxy acetic acid, 1-naphthylacetic acid, 2-naphthoxyacetic acid, and 2-naphthylthioacetic acid) were studied. The effective rate constants in experiments of alkaline hydrolysis decreased as the molecular mass of the polymer increased, the content of acid hydrophobic residues in polymer increased, and the acidity of the acid decreased. Data on the elucidation of stucture versus release-rate relationships in polymeric esters of plant growth regulators will further aid the preparation of novel delivery systems with optimal biological activity.

1. Introduction

Phytoactive polymers, polymeric derivatives of plant growth regulators (PGRs), are biologically active after releasing the low-molecular regulator as a result of the hydrolysis of the chemical bond linking the regulator to the polymer carrier [1–10]. In spite of the series of works in this direction, the effect the chemical structure of phytoactive polymers on their ability for hydrolysis was not sufficiently studied [11–14]. At the same time such knowledge may prove useful in the development of novel highly efficient preparations for plant growing.

The aim of the present study is to reveal how the rate of hydrolysis is correlated with structure of water soluble polymeric esters of the above mentioned aryl-containing carboxylic acids of the auxin type, containing various side groups (lyophilizing groups) imparting to the polymer solubility in water on hydrophilicity.

To address these questions we studied polymeric esters of 2,4-dichlorophenoxy acetic acid (2,4-D), 1-naphthylacetic acid (NAA), 2-naphthoxyacetic acid (NOAA) and 2-naphthylthioacetic acid (NTAA) (Fig. 1A–C). The polymeric esters of PGRs were prepared by the reaction of the acrylamide-vinyl-2-chloroethyl ether (90.5 : 9.5) with the potassium salts of the acids 2,4-D, NAA, NOAA, and NTAA (Method A); by co-polymerization of the allylic ester of 2,4-D (AE 2,4-D) with acrylamide, vinylpyrrolidone or acrylic acid (Method B); and by acylation of polyvinyl alcohol of different molecular weight with acyl chlorides of 2,4-D and NAA (Method C).

The selection of these polymeric derivatives of PGR's enabled us to examine the effect of different side groups of their structure (e.g., the type of the side lyophilizing groups, the amount of the PGR residues in the polymer, the molecular weight of the carrier polymer, and the structure of PGR) on their hydrolysis.

2. Materials and methods

2.1. Starting compounds

2.1.1. Method A

Copolymers of acrylamide with 2-chloroethyl-vinyl ether (CEVE) containing 9.5% CEVE units, of 24×10^3 M_w, were prepared by polymerization of the monomers in isopropanol in the presence of azodiisobutironitrile (AIBN) [15]. The synthesis of polymeric esters of 2,4-D, NAA, NOAA and NTAA was carried out by the reaction of acrylamide-CEVE copolymer with the

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potassium salts of these acids in DMSO [15] (Fig. 1A). The polymer esters used contained 0.7–0.8 base-mol% of the PGR (Table I). The content of the PGR residues in copolymers was calculated spectrophotometrically by measuring the absorbance of the polymers at $\lambda = 282$ nm for NAA, 271 nm for NOAA, 252 nm for NTAA, and 284 nm for 2,4-D in DMSO and water (Specord UV-VIS instrument, Germany).

2.1.2. Method B

The copolymers of 2,4-D allylic ester and acrylic acid were synthesized in isopropyl alcohol in the presence of AIBN as an initiator (Fig. 1B). The copolymer was

TABLE I The effective hydrolysis rate constants of PGR (NAA, NOAA, NTAA, 2,4-D) polymeric esters prepared by reaction of potassium salts of PGRs with acrylamide-CEVE copolymer (pH = 10.0, T = 320 K) (Fig. 1, method A)

		PGR polymeric ester	
PGR	PGR (p Ka)	(Mol%)[a]	k^0_{ef} (dm^3 mol^{-1} s^{-1})[b]
NAA	5.12	0.81	0.58 ± 0.02
NOAA	3.38	0.76	7.47 ± 0.25
NTAA	3.13	0.71	9.80 ± 0.37
2,4-D	2.94	0.78	12.90 ± 0.28

[a] The content of the PGR units with carboxylic acid residues.

[b] k^0_{ef} is the effective second-order rate constant corresponding to the initial reaction stage.

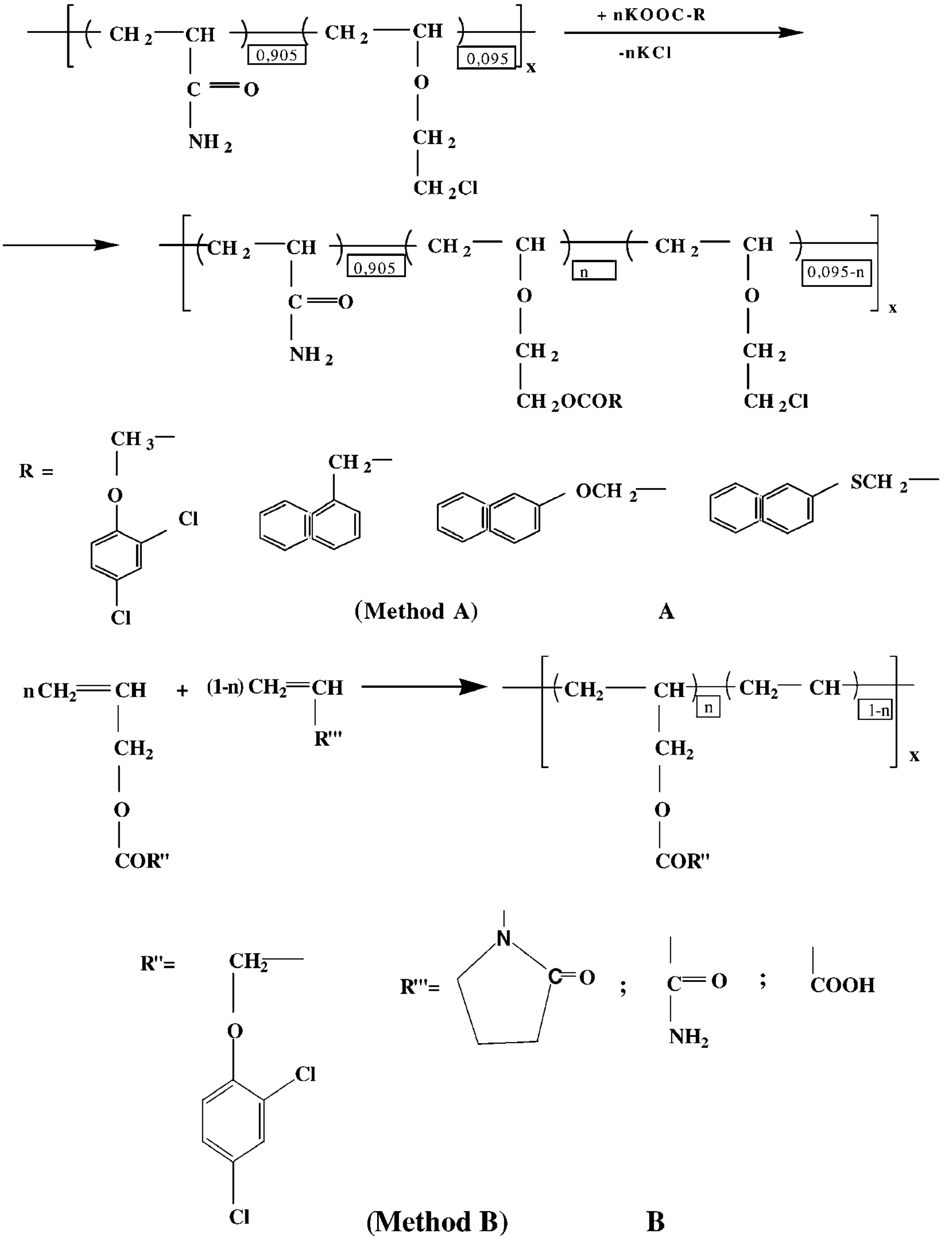

Figure 1 Methods of preparation (A, B, C) and chemical structures of the synthesized polymeric esters of carboxyl-containing plant growth regulators of 2,4-dichlorophenoxyacetic acid (2,4-D), 1-napthylacetic acid (NAA), 2-naphthoxyacetic acid (NOAA) and 2-naphtylthioacetic acid (NTAA). Symbol in the designation of the ring molar fraction indicates on its statistical distribution in the polymer chain. (*Continued.*)

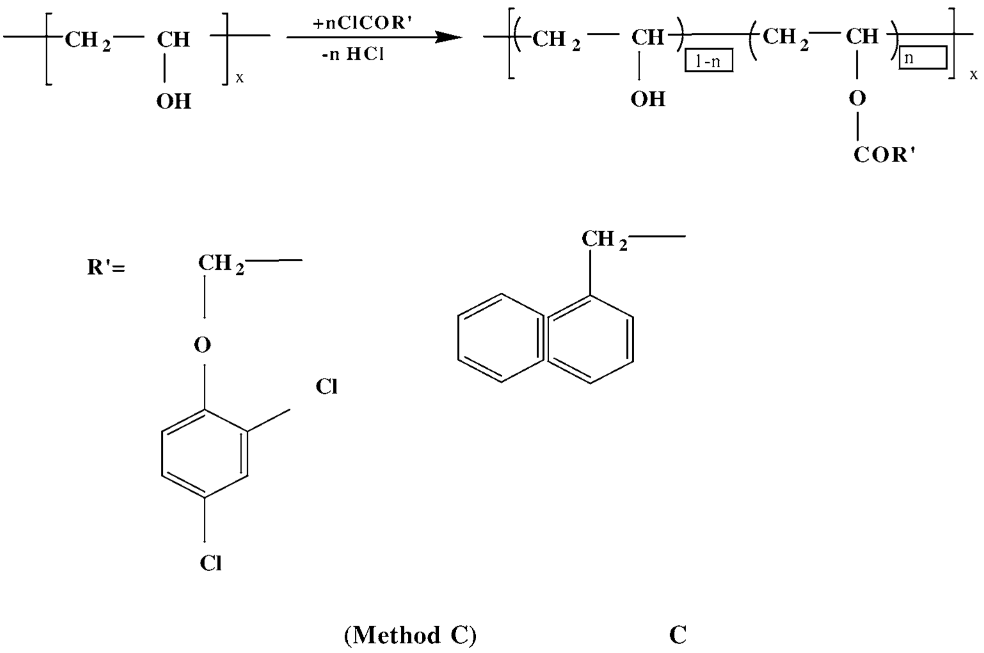

Figure 1 (*Continued.*)

isolated by filtration, followed by washing with pentane and drying in vacuum to a constant weight. The copolymers with acrylamide and N-vinylpyrolidone were prepared in dioxane respectively in a similar way. The acrylamide copolymer precipitated during the synthesis, whereas the N-vinylpyrolidone copolymer was precipitated in diethyl ether. In order to prepare copolymers with desired molecular masses and with certain PGR residues co-polymerization was performed by varying the ratio of each monomer in the reaction mixture. The resulting copolymers were subjected to fractional precipitation and the precipitated fractions were separated by centrifugation.

The molecular weight of the copolymers at low concentrations of the substitutes was measured by viscometry using an Ostwald viscometer (with dilutions in the range of concentrations 1–15 g dm^{-3}) according the formula $[\eta] = (6.31 \times 10^{-3})\ M_w^{0.8}$ for the acrylamide copolymer (in water) [16], $[\eta] = (1.3 \times 10^{-2})\ M_w^{0.68}$ for the N-vinylpyrrolidone copolymer (in water) [17] and $[\eta] = (76 \times 10^{-5})\ M_w^{0.5}$ for the acrylic acid copolymer (in dioxane) [18]. The content of the PGR residues in copolymers was calculated spectrophotometrically.

2.1.3. Method C

Polyvinyl alcohol of grades 7/1, 16/1 and 40/2 ("Plastpolymer," Yerevan) prepared by alkaline hydrolysis and purified by methanol extraction was used. The content of acetate groups in these species was 0.78, 0.93 and 1.21 respectively. The molecular mass, measured viscometrically according to the formula $[\eta] = (140 \times 10^{-3})\ M_w^{0.6}$, was 12.0×10^3, 29.0×10^3 and 65.0×10^3 respectively [14, 16].

Polyvinyl esters were synthesized by acylation of PVA with acyl chlorides in dimethylacetamide accordingly to procedure described elsewhere [19] (Fig. 1C). The amount of PGR residues coupled to the polymer was regulated by varying the amount of acyl chloride in the reaction mixture. With an increase in the content of conjugated acidic residues, IR spectra of polyvinyl esters showed an increase in intensity of the bands due to the ester carbonyl at 1740 cm^{-1} (for 2,4-D) and 1720 cm^{-1} (for NAA). The amount of the PGR content in the polymers was also determined also by UV spectroscopy.

2.2. Hydrolysis studies

A weighed amount of 0.01 g polymer powder sieved through a sieve of 0.1 mm pore size was discharged into a small jacket flask connected to a thermostat and 0.5×10^{-3} dm^3 of distilled water were added. The suspension was left stirring at 50°C for 3 h with a magnetic stirrer. The polymer species with the low content in active ingredient (not more than 2.0–2.5 mol%) were dissolved, whereas the polymer species with a higher content in acid residues remained in the solid phase. The temperature in the jacket was brought to the desired values and 4.5×10^{-6} m^3 of a buffer solution (pH = 10.0) were added.

The amount of acid released as a result of the hydrolysis was detected by HPLC (LKB instrument equipped with a Bondapak C18 column from Waters). The released acids were detected at the wave length corresponding to each acid and appropriate retention times. A mixture of ethanol, water and acetic acid (145 : 130 : 15 volume ratios) was used as the eluent.

The hydrolysis ability was estimated by measuring the effective rate constant corresponding to initial moment of reaction (k_{ef}^{0}), calculated according to the second-order. The experimental accuracy was assessed in several series of experiments. The values of hydrolysis degree versus time represent the mean of five readings from parallel experiments and its CV ranged from 2.5–4.5%.

3. Results and discussion

The study of the hydrolysis of the synthesized polymeric esters revealed that depending on the content of

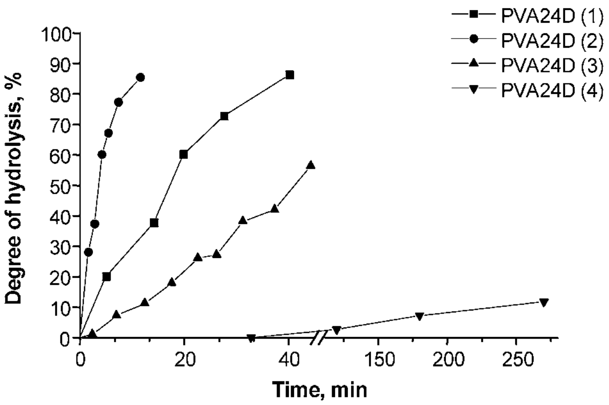

Figure 2 Degree of hydrolysis process versus time for the 2,4-D esters with poly (vinyl alcohol) (PVA24D(1–4); $M_w = 12 \times 10^3$; Fig. 1C). The content of 2,4-D in the polymers: (1) 1.3, (2) 3.0, (3) 14.8, and (4) 32.7 base-mol%. Initial concentration of ester groups ($[E]_0 \times 10^3$): (1) 0.98, (2) 3.30, (3) 3.60, and (4) 3.54 base-mol dm^{-3}. $T =$ (1) 313, and (2–4) 363 K.

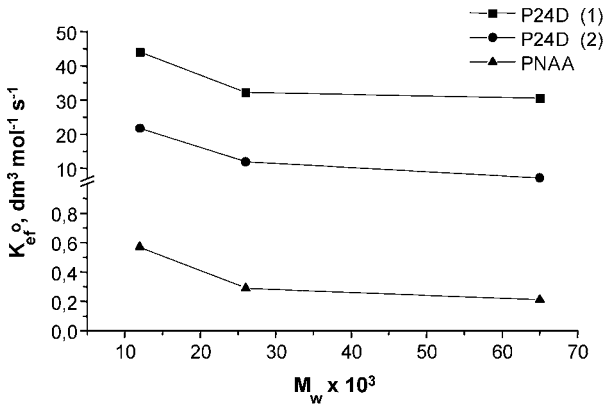

Figure 3 The effect of molecular weight (M_w) on the effective hydrolysis rate constant (k^0_{ef}) of polyvinyl esters of 2,4-D and NAA (Fig. 1A) $T = 363$ K. Initial concentration of ester groups. $[E]_0 = (2.6 - 3.8) \times 10^{-3}$ base-mol dm^{-3}. Content of 2,4-D residues; 0.68 – 0.93 (1), 2.4 – 3.0 (2); and of NAA 2.1 – 2.9 base-mol%.

aromatic (2,4-D; NAA) residues in the polymer structure (Fig. 2), the kinetic curves showed either a saturation plateau (for water soluble polymers with low content in acid residues; Fig. 2, PVA24D (1) and (2), or were S-shaped curves of different steepness (for insoluble polymers; Fig. 2, PVA24D (3) and (4)). The S-shape pattern of the kinetic curves may apparently be explained by the fact that hydrophilicity and solubility of initially insoluble polymeric esters gradually increase as the content of aromatic PGR residues decreases. In water-soluble polymers with a low content in aromatic residues, the current rate constants remained the same even at rather high conversions (Fig. 2, PVA24D (1)).

Because the alkaline hydrolysis (pH = 10.0) is studied, the reaction rate constants were calculated according to the second-order equation recommended for the hydrolysis of esters under known conditions and a known scheme of the reaction involving nucleophilic attack of an OH$^-$ group to the carbon of a carbonyl group of the ester. The validity of this equation was verified by determining the partial reaction orders with respect to reagents, which were found to be close to the value of one. In order to eliminate the effects due to the variations of the polymer structure during the reaction, so as to be able to compare the hydrolytic activity of different polymeric esters, k^0_{ef} values corresponding to the initial stage of the hydrolysis reaction were used (14,15,19).

3.1. Effect of the type of carboxylic acid

In Table I the reaction rate constants k^0_{ef} are shown for the polymeric esters of various carboxylic acids prepared after the reaction with the acrylamide-CEVE (AACEVE) copolymer. As it is seen, under these conditions the hydrolysis is predominantly controlled by the structure of the carboxylic acid, which as in the case a low molecular mass esters affects the acidity and the stability of the hydrolyzed bond. The experimental data indicates that the hydrolysis rate markedly increases as the acidity of the acid increases. Among the compounds studied the polymeric ester of the strong acid 2,4-D showed the highest capability to undergo hydrolysis, whereas the lower capability to undergo hydrolysis was observed in the polymeric ester of the weak acid NAA (Table I).

A similar observation is noted for the hydrolysis of polyvinyl esters of 2,4-D and NAA. In this case the k^0_{ef} values of the hydrolysis of 2,4-D polyvinyl esters {P24D(1)(2)} were higher than those of the hydrolysis of NAA-containing polymer (Fig. 3).

3.1.1. Effect of molecular weight

The interplay between the hydrolysis of phytoactive polymers and their molecular weight has been described in the literature. McCormick *et al.* examined the kinetic curves for alkaline hydrolysis of water insoluble copolymers of 2-(1-naphthylacetyl)acrylate and acrylamide with 30 mol% of aromatic units [12]. It was demonstrated in this study that the rate of the hydrolysis decreased as the molecular weight of the polymer increases. In the range of 55×10^3 to 90×10^3 the decrease was not significant. However the decrease in the hydrolysis rate was significant at a M_w of 180×10^3.

In the present study the effect of the molecular mass of the polymers on their hydrolysis was examined with polyvinyl esters of NAA and 2,4-D of a different content in aromatic residues, the lower content being 1 mol%. In this case the effect of the co-polymers on the conformation of the polymer could be ignored and the effects of the molecular weight could be identified. As it is seen in Fig. 3, in all cases the k^0_{ef} value decreases as the molecular mass increases from 12.0×10^3 to 29.0×10^3. One explanation is that decrease in k^0_{ef} is observed in these cases where a certain molecular mass M_w (η_{sp}) and macromolecular conformation is achieved. Such a conformation of the macromolecular structure results by transition from an extended randomly curved rod to a random coil. The occurrence of hydrolysis inside a such coil is sterically hindered [20].

A similar picture was obtained from the experiments on the water-soluble copolymers of AE 2,4-D with acrylic acid (Fig. 4). In this case the hydrolysis of the copolymers containing small amounts of substitutes was studied. The demonstrated influence on hydrolysis of the molecular weight for this entire range of

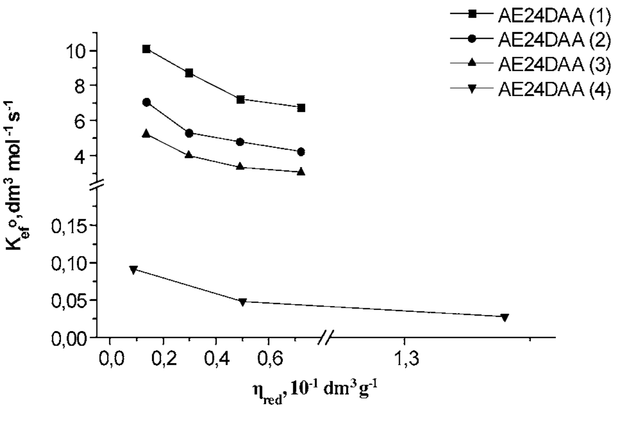

Figure 4 The effect of reduced viscosity (η_{red}) on the hydrolysis rate constant (k_{ef}^{0}) of AE 2,4-D copolymers with (1–3) acrylamide and (4) acrylic acid (Fig. 1B) $T = $ (1) 308, (2) 303, (3) 298, (4) 343 K. Concentration of esters groups $[E]_0 \times 10^3$: (1 – 3) 1.3 – 1.4 and (4) 4.7 – 5.6 base-mol dm^{-3}. The content of AE 2,4-D units in copolymers: (1–3) 0.49 – 0.51 and (4) 3.7 – 4.7 base-mol%.

molecular weight values is very important as this is generally associated with the desired properties of biocompatibility and the low toxicity for polymers.

3.2. Effect of amount of PGR residues

The data obtained for the effect of the amount of PGR residues on hydrolysis rate for water insoluble polymers may be partially interpreted by the occuring steric effects. Steric effect hinders the migration of OH$^-$ to the hydrolysable group and the subsequent migration of the cleaved acid residue from the transition complex [12].

As it is seen in Fig. 6, increasing amount of 2,4-D acid residues incorporated into the polyvinyl alcohol upto 10 mol% leads to a dramatic decrease in k_{ef}^{0} values. Apparently this is the result of high hydrophobicity and low solubility of the polymer during the hydrolysis reaction. In particular, at a certain content in aromatic residues the dissolved macromolecule collapses because of the increasing hydrophobic interactions.

This observation is confirmed by measurements of the intrinsic viscosity ($[\eta]$) of 2,4-D polyvinyl esters of different molecular weights and different content in aromatic residues. The measurements were performed in DMSO, which dissolved all samples of polyvinyl esters (Fig. 5). As it is seen, increasing the content in aromatic residues leads to a lower $[\eta]$ especially at a low degrees of substitution. Moreover, this effect is more pronounced for polymers with high molecular weight (Fig. 5, PVA24D (1) and (2)). Figs 4 and 5 suggest that for the polyvinyl esters studied, the changes in conformation of the macromolecules in solution become noticeable at a greater content in aromatic residues than 1 mol%. The highest conformational compacting takes place at 10 mol% content of aromatic residues.

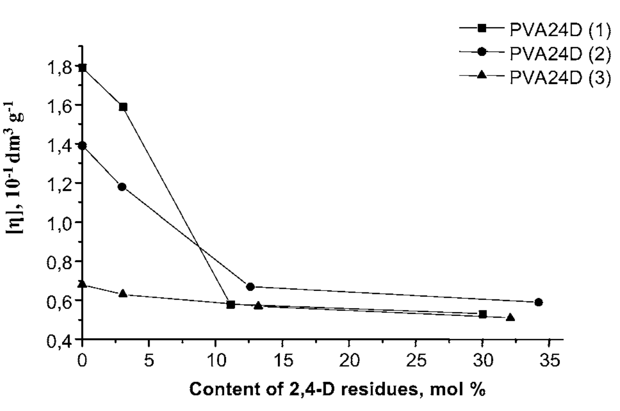

Figure 5 Plot of intrinsic viscosity ($[\eta]$) vs. content of 2,4-D residues in polyvinyl esters of 2,4-D (Fig. 1C); $M_w \times 10^{-3}$: (1) 65, (2) 29, (3) 12.

TABLE II The values of effective rate constant (k_{ef}^{0}) for the hydrolysis of 2,4-D polymeric esters (Fig. 1) (pH 10.0, $T = 312$ K)

Polymeric ester of 2,4-D	Content of units with 2,4-D, mol%	M_w, kD	k_{ef}^{0} (dm^3 mol^{-1} s^{-1})
Poly(vinyl alcohol) 2,4-D ester (Method C) (PVA24D)	0.72	29	5.20 ± 0.35
AE 2,4-D – acrylic acid copolymer (Method B) AE24DAAC	0.68	25	0.11 ± 0.03
AE 2,4-D – acrylamide copolymer (Method B) AE24DAAM	0.51	26	8.42 ± 0.43
AE2,4-D–N-vinylpyrrolidone coplr (Method B) AE24DNVP	0.75	27	5.95 ± 0.28

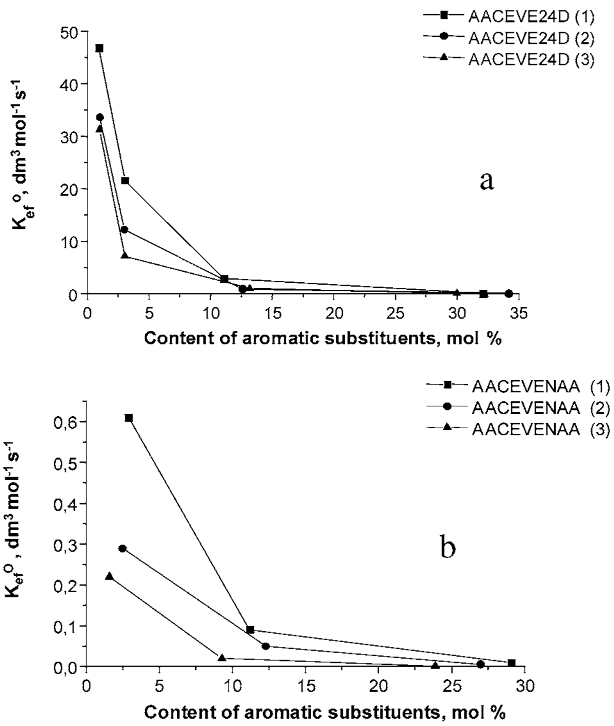

Figure 6 Plots of the effective hydrolysis rate constant (k_{ef}^{0}) vs. content of 2,4-D (a) and NAA (b) residues for the polyvinyl esters of AACEVE copolymers (Fig. 1A) $T = 363$ K. Concentration of esters groups $[E]_0 = 10^3$ (a) 3.3 – 3.6 and (b) 3.3 – 3.8 base-mol dm^{-3}; $M_w \times 10^{-3}$: (1) 12, (2) 29, (3) 65.

3.3. Effect of the type of the side-lyophilizing group of polymer

The rate constants of the hydrolysis of AE 2,4-D copolymers and various hydrophilic copolymers (acrylamide, N-vinylpyrrolidone, acrylic acid) are listed in Table II. The rate constant of the hydrolysis of the polyvinyl ester of 2,4-D is also shown. All the above complexes are water-soluble. They contain one and the same regulator, their molecular mass is similar and their content in active ingredient is almost equal. To minimize the influence of compacting into macromolecular coil, which was observed when side aromatic groups were introduced into water soluble polymers, we used polymeric esters containing sequential units with acid residues (0.7–0.8 mol%). This amount of hydrophobic substitute was close to the amount of specific labels (fluorescent, paramagnetic, etc.) that are commonly introduced into polymers. In this case the effect of different structures of the side-lyophilizing group upon the hydrolysis of these polymers can be directly accessed when studying the ability of the polymeric esters to be hydrolyzed.

As it is seen from Table II, the values of the k_{ef}^{0} for the derivatives of acrylamide, and N-vinylpyrrolidone are similar. This indicates that the structure of the side groups (primary and cyclic amide) and the difference in the macromolecular coil extent insignificantly affects the hydrolysis of the active compound. It is possible that the somewhat greater hydrolysis constant of the acrylamide copolymer is caused by the interaction between an ester and neighboring amide groups forming a hydrogen bond and an intermediate imide structure [21].

For the acrylic acid copolymer, which occurs as salt, under the conditions of alkaline hydrolysis the values of k_{ef}^{0} were remarkably lower as compared to the other polymeric esters. Although, hydrolysis was conducted at 343 K, that is at a higher temperature. The lower values of k_{ef}^{0} for the acrylic acid copolymers are also noted in Fig. 3, where the effect of the molecular masses of the polymers on hydrolysis is seen. Possibly the lower rate of hydrolysis of the acrylic acid copolymers in alkaline solution is attributed to the negative charged carboxylate group. The latter is present in neighboring units of the copolymer and hampers the diffusion of OH ions, which are important in the hydrolysis reaction (Fig. 1). Apparently the underlying mechanism of hydrolysis accompanied with the involvement of the carboxylate group in the catalysis and the formation of anhydride as an intermediate, also noted for a number of esters of polyacrylic acids [22], cannot be overemphasized.

4. Conclusion

The present study revealed the relationship between the hydrolytic release rate of the low molecular weight regulator (PGR) and the macromolecular structure of the phytoactive polymers containing the PGR.

Particularly, the considered series of phytoactive polymers demonstrated that the content of hydrophobic PGR residues in polymer plays significant role changing the values of rate constants on several orders. The presence in polymer of side group neighbored to hydrolyzed group and other factors of bonded PGR structure (e.g., the acid strength in this case) can also play an important role. On the other side, the molecular weight of polymers (in the studied range of values) does not have a dramatic effect on the hydrolysis rate.

The results obtained support the fact that the activity of the polymeric PGRs is due to their release from the polymers. This conclusion is essential because the determination of the hydrolysis rate constant k_{ef}^{0} can be measured easily under laboratory conditions and the hydrolysis rate constants of different polymers measured *in vitro* may correspond to the real conditions with physiological pH and temperature and participation of enzymes in the hydrolysis reaction.

The results of this study can be used for the synthesis of new phytoactive polymers with a required hydrolysis rate and during the preparation of other types of macromolecular systems with controlled release action, for example, drug polymers.

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