Radiation-induced Chemical Synthesis of Carriers of Biologically Active Compounds: Copolymers of N-Vinyl-2-pyrrolidone with Allyl Alcohol and Allyl Amine

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ABSTRACT

Radiation-induced copolymerization is a very promising method for the synthesis of polymers with the required combination of properties. It provides the possibility of controlling the copolymerization process and, thus, to obtain copolymers with the desired composition and molecular weight characteristics as well as copolymers with comonomers that are difficult to polymerize. This work deals with the characterization of radiation-induced copolymerization of N-vinyl-2-pyrrolidone (VP) with such comonomers: allyl alcohol and allylamine in bulk, in ethanol or isopropanol at 320 K and at an irradiation dose rate of 2 Gys. It was established that by changing the characteristics of the irradiated medium (irradiation dose, composition and concentration of the initial monomer mixture and solvent nature) it is possible to obtain water-soluble reactive copolymers of VP with allyl alcohol and allylamine. Their molecular weights, $\bar{M}_w$, are between 10-68 x 10^3, their molecular weight distribution is relatively narrow ($\bar{M}_w / \bar{M}_n=1.4-2.8$) and they contain 12.8-24.3 mol % of functional groups. The structure of these copolymers was confirmed by functional analysis and IR spectroscopy. Acute toxicity of these functional copolymers was investigated. Some of these copolymers in their principal characteristic meet the requirements that should be fulfilled by synthetic non-biodegradable polymers, modifiers of biologically active compounds. Specific examples are reported in using them as carriers of biologically active compounds with fungicidal activity.

Key Words: radiation, copolymerization, copolymer-carriers of biologically active compounds, N-vinyl-2-pyrrolidone, allyl alcohol, allyl amine

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INTRODUCTION

The copolymerization of N-vinyl-2-pyrrolidone (VP) with allyl monomers is a readily available and universal method for obtaining potential copolymer-carriers of biologically active compounds (BAC). They contain a wide range of functional groups, exhibiting limited low molecular weights, narrow MWD, and low sequence length of the reactive unit, which facilitates the reaction of this copolymers with BAC.

VP copolymers with allyl alcohol (AA), formula 1 in Scheme I containing primary hydroxyl groups and VP copolymers with allyl amine (AAm), formula 2 in Scheme I containing primary amino groups separated from the main copolymer chain by the -CH$_2 -$ group are of particular interest among VP copolymers with functional allyl monomers.

It is very convenient to use these copolymers for modifying BAC, as they contain chloroalkyl, aldehyde, carboxylic, activated ester, and other reactive groups.

Copolymer 1 with ([m$_{2}$]=9.2 mol%) and co-

CH$_2$-CH

H$_2$C

N

C=O

H$_2$C-CH$_2$

m$_1$

CH$_2$-CH

H$_2$C

N

C=O

H$_2$C-CH$_2$

m$_1$

OH

m$_2$

CH$_2$-CH

H$_2$C

N

C=O

H$_2$C-CH$_2$

m$_1$

NH$_2$

m$_2$

Scheme I

m$_1$, m$_2$ - molar composition of the copolymer, %; m$_1$+m$_2$=100

decomposition of polymer 1 ([m$_2$]=12.7 mol%) obtained by free-radical copolymerization of VP with AA and VP with AAm in a solvent in the presence of AIBN have been described in the literature [1]. However, the yield of the copolymers has not been reported. Moreover for BAC modification often water-soluble polymer carriers with higher functional groups content of 15–20 mol% are often required.

It has been possible to obtain [2] VP–AAm.HX copolymers in 98–99% yield in the copolymerization of VP with AAm salts in water in the presence of a specially synthesized water-soluble initiator, 2,2-azobis (2-amidinylpropane) dichloride. These copolymers contained 10.8–27.1 mol% of AAm.HX units, their intrinsic viscosity ranged from 0.1 to 1.08 and their molecular weight distribution was not investigated.

However, it seemed advisable to study direct single-stage copolymerization of VP with AAm in the base form in an organic solvent by using a readily available initiator AIBN.

Our data (Table 1) show that copolymers with this composition (15–20 mol% allyl monomer) can be obtained by free-radical copolymerization of VP with AA and VP with AAm initiated by AIBN only in very low yield. This is due to degradation chain transfer to the allyl monomer. Allyl radicals are stable to initiate polymerization. Therefore, during the act of chain transfer chain termination occurs.

In order to intensify the process of copolymers (1 and 2) preparation, the method of radiation-induced initiation of VP copolymerization with AA and with AAm was used. Radiation-induced copolymerization is relatively often employed to synthesize polymer BAC carriers [3–5]. The following advantages of radiation-induced initiation of polymerization in the synthesis of polymer BAC carriers should be mentioned. First, relatively pure polymers without initiator residue are obtained. Second, the molecular weight characteristics of the copolymers can be controlled rather easily by changing the dose rate of radiation and by other procedures. Third, in some cases (particularly our case) polymer yield is much higher than that when chemical substances are used for initiation. Finally, radiation-induced initiation makes it possible to use
Table 1. Copolymerization of VP with AA and VP with AAm in presence of AIBN at 343 K.

<table>
<thead>
<tr>
<th></th>
<th>Solvent</th>
<th>[M₁+M₂] mass (%)</th>
<th>AIBN mass (%)</th>
<th>[M₀] mol (%)</th>
<th>Yield (%)</th>
<th>[M₂] mol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allyl alcohol</td>
<td>Dioxane</td>
<td>40</td>
<td>0.5</td>
<td>25</td>
<td>50</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>Dioxane</td>
<td>40</td>
<td>0.5</td>
<td>50</td>
<td>20</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>Dioxane</td>
<td>40</td>
<td>0.5</td>
<td>65</td>
<td>3</td>
<td>18.6</td>
</tr>
<tr>
<td>Allylamine</td>
<td>in bulk</td>
<td>100</td>
<td>0.8</td>
<td>15</td>
<td>46.4</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.8</td>
<td>20</td>
<td>44.2</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.8</td>
<td>40</td>
<td>26.4</td>
<td>15.2</td>
</tr>
</tbody>
</table>

such polymers that do not easily undergo (co)-polymerization.

The efficiency of radiation-induced chemical synthesis of copolymers (1 and 2) was evaluated and the composition, toxicity and molecular weight characteristics of these copolymers were determined. It is known [6], that these characteristics determine to a considerable extent the possibility of using water-soluble synthetic non-biodegradable polymers for BAC modification.

It is well known [7] that biologically active polymers (BAP) can be obtained not only by attaching BAC to polymer carrier, but also by the polymerization unsaturated BAC derivatives. For this purpose radiation-induced polymerization is also successfully used. References 8, 9 report that water-soluble polymers with hypotensive and hemostatic activity have been obtained through (co)polymerization, induced by γ-irradiation of acrylic or methacrylic esters of lupine and anabazine alkaloids. Taking as an example these BAP the authors studied the relationship of biological properties of the polymers to their macromolecular characteristics: molecular and compositional inhomogeneity and composition (for copolymers).

EXPERIMENTAL

VP was purified by two-fold vacuum distillation over KU-2 cation-exchange resin in the presence of hydroquinone. AA and AAm were distilled twice over molecular sieves. Physico-chemical constants of the monomers were in agreement with the data from literature. Monomer purity was confirmed by gas-liquid chromatography on a Tswett-6 chromatograph with a flame ionization detector. The carrier gas was nitrogen and the sorbent contained 5% of polyethylene glycol adipinate and 10% of dinonyl-phthalate on zeolite 545. Heptyl alcohol was used as standard. Chromatographically pure VP, AA, and AAm were employed.

The radiation-induced copolymerization of comonomers was carried out in ethanol or isopropanol in degassed sealed ampouls placed in the channel of the MRKH-γ-20 unit. The 60Co radiation dose rate was 2.0 Gy/s and channel temperature was 320 K. The composition of copolymer 1 was determined by the acetylation method [10] and that of copolymer 2 by complexation with trinitrobenzenesulphonic acid in an aqueous solution at pH 8.0 by the method described in ref. [11].

Intrinsic viscosity of copolymers of type 1 was measured in water and that of copolymers of type 2 was measured in a 0.1 N solution of sodium acetate in water in an Ubbelohde viscometer at 298±0.01 K. Mark-Kuhn-Houwink equations described in refs. 12 and 13 were used to determine viscosity-average molecular weights (Mᵥ) of copolymers (1 and 2), respectively. Weight-average and number-average molecular weights and MWD of copolymers (1 and 2) were determined by GPC in DMF. A system of chromatographic styrogel microcolumns (Waters) with porosities of 10³, 10⁴ and 10⁵ nm was used. The chromatograph was calibrated using narrow-disperse polystyrene (PS) samples (Waters).

Proceeding from the principle of the Benoit universal calibrations [14], calibration dependence was calculated for poly-N-vinyl-2-pyrrolidone (PVP). The
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following ratios of intrinsic viscosity to molecular weight in DMF were used: \([\eta] = 5.49 \times 10^{-4} \cdot M^{-0.85}\) (for PVP) and \([\eta] = 1.23 \times 10^{-4} \cdot M^{-0.60}\) (for PS). When universal calibration was applied for calculating the MWD of VP copolymers it was assumed that relatively low M_2 units content in the copolymer and the absence of microblocks of M_2 units make it possible to use the calibration found for PVP [15].

IR Spectra in the film cast from solutions in chloroform were recorded with a Specord IR-75 spectrophotometer. The IR spectrum of the VP–AA copolymer was 1650 cm\(^{-1}\) (\(\nu_{C=O}\) of the VP unit), 1140 and 1270 cm\(^{-1}\) (\(\nu_{C-O}\) of the AA unit), and 3400 cm\(^{-1}\) (\(\nu_{OH}\)). The IR spectrum of the VP–AAm copolymer was: 1630 cm\(^{-1}\) (\(\nu_{C=O}\) of the VP unit), 3430 cm\(^{-1}\) and 3370 cm\(^{-1}\) (\(\nu_{NH_2}\)), and 1220 cm\(^{-1}\) (\(\nu_{C-N}\) of the AAm unit).

The toxicity of the copolymers (1 and 2) was evaluated in experiments on white mice with intra peritoneal administration. The values of LD_{50} were calculated by Kerber’s method.

RESULTS AND DISCUSSION

It was established (curve 1 in Figure 1a) that radiation-induced copolymerization of VP (M_1) with AA (M_2) in ethanol in the molar ratio of monomers in

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Kinetics of radiation-induced copolymerization of (1) VP with AA and (2) VP with AAm in ethanol at [M_1]:[M_2]=85:15 mol %, at [M_1+M_2]= (a) 30 mass% and (b) 3 mass%.

the initial mixture VP:AA = 85:15 and at their mass fraction [M_1 + M_2] = 30 mass% and at a relatively high radiation dose (D) proceeds at a high rate. At radiation doses of 30 and 50 kGy the yield of copolymer 1 is 87 and 98%, respectively and radiation-chemical yields (G) are 1550 and 2800 molecules per 100 eV, respectively. As can be seen from Figure 2, the curves describing the dependency of the yield of copolymer 1 at a constant dose on the mass fraction of the initial monomer mixture pass through the maximum at [M_1+M_2]=30%. The lowest yield is observed for copolymerization in bulk ([M_1+M_2]=100%).

Higher copolymerization rate in solutions than that in bulk is caused by the participation in the initiation of free radicals formed during solvent radiolysis. In concentrated solutions a smaller number of active centres are formed. Moreover they disappear more rapidly because degrading chain transfer increases with the viscosity of the irradiated system. This leads to the observed decrease in polymerization rate at [M_1+M_2]=30%. Lower copolymerization rate of VP with AA in isopropanol than that in ethanol (curve 2

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Dependence of the yield of VP–AA copolymers obtained by copolymerization in (1) ethanol and (2) isopropanol on mass fraction of the initial monomer mixture with the molar composition of VP:AA=85:15; D=50 kGy.
in Figure 2) is due to the fact that isopropanol is a strong transfer agent of the growing polymer chain [12] decreasing the copolymerization rate of copolymer I and its molecular weight.

Other irradiation conditions being equal, AAm copolymerizes with VP at a lower rate than AA (Figure 1, curve 2). In this case at D=30 and D=50 kGy the yield of copolymer 2 attains 70 and 88%, respectively, and radiation-chemical yields are 1250 and 1500 molecules per 100 eV. The lower rate of radiation-induced copolymerization of VP with AAm is particularly pronounced in dilute solutions of comonomers at [M1+M2]=3 mass% (Figure 1b, curve 2 as compared to curve 1). These data are in agreement with the results of Dolmatov and Polak [17] who have studied radiation-induced homopolymerization of AA and AAm. These authors have shown that AAm characterized by G value equal to 15 at D=18 kGy, whereas a much higher G value was found for AA. It is equal to 35 molecules per 100 eV even at a lower dose (D=2.4 kGy). This phenomenon is evidently due to the fact that for the allyl amine the process of degrading chain transfer is caused not only by the abstraction of the hydrogen atom in the α-position to the double bond but also by the abstraction of mobile hydrogen atoms in the primary amino group. Taking into account the low reactivity of AAm in radiation-induced polymerization, the further study of radiation-induced copolymerization of VP with AA and with AAm was mainly carried out at a dose of 200 kg.

The investigation of the dependence of copolymers (1 and 2) compositions, their yield, and Mv on the composition of the initial monomer mixture made it possible to establish the common features of radiation-induced free-radical copolymerization of these monomers in solution. As can be seen in Figures 3 and 4, the yield and Mv of the copolymers decreased markedly with increasing molar fraction of the allyl monomer in the initial mixture. This results from increasing probability of kinetic chain termination with the participation of the allyl monomer and from increasing quantity of oligomer products. As for the compositions of copolymers 1 and 2, they increase monotonically with increasing allyl monomer content in the initial monomer mixture (Figures 3b and 4b). This dependence is more pronounced for the more active comonomer, AA. Thus, at [M2]=50 mol%, D=200 kGy, and [M1+M2]=30 mass%, copolymer 1 containing 31 mol% of AA units and copolymer 2 containing 23 mol% of AAm units are formed.

However, it should be noted that the yield of copolymers of type 1 with [M2]=15–20 mol% is 86–98% (Figure 3b). The viscosity-average molecular weights of these copolymers are 32,000–45,000. Copolymers of type 2 with the same composition are formed in 63–72% yield and Mv=12,000–19,000. These data show that copolymers 1 and 2, which meet the requirements of composition and low molecular weights that should be met by BAC polymer carriers, can be obtained by radiation-induced chemical synthesis in high yields.

With a constant composition of the initial monomer mixture and constant radiation dose, the molecular weights can be effectively controlled by varying the concentration of the initial monomer mixture [M1+M2] before γ-irradiation. Figure 5 shows...
that the decrease in \([M_1+M_2]\) to 3–10 mass% depending on the alcohol used, leads to the preparation of copolymers of type 1 with limited low molecular weights: 13,000–33,000. In the case of copolymers of type 2 (Figure 5(a)), low molecular weight copolymers with \(M_\text{w}=10,000–25,000\) are obtained at \([M_1+M_2]=5–15\) mass%. The decrease in the degree of polymerization when comonomer solution is diluted to \([M_1+M_2]=3–15\) mass% before \(\gamma\)-irradiation is due to the effect of solvent radiolysis products and to the redistribution of the absorbed radiation energy between the molecules of the monomer and the solvent.

Finally, in this application of copolymers 1 and 2 which are not biodegradable polymers but must be easily released from the living organism must not contain fractions with \(M_\text{w}>80,000\), it is very important that they should have not only low molecular weights 20–40\(\times\)10\(^3\) but also narrow MWD. Radiation-induced polymerization of VP with AA and VP with AAm solves this problem. As it follows from Table 2, the degree of polydispersity of these copolymers also depends on the concentration of the irradiated solution. At \(D=50\) kGy and \([M_2]=15\) mol%, with decreasing \([M_1+M_2]\) value from 30 to 3 mass%, the values of \(M_\text{w}/M_\text{n}\) for copolymer 1 decrease from 2.84 to 1.86 in ethanol and 1.37 in isopropanol, respectively. At higher dilution the number of growing reactive chains increases. This is manifested in an increase of radiation-chemical yield of initiation and leads to the formation of short chains and narrower MWD of copolymers.

It is interesting to point out that copolymers 1 with narrow MWD are formed in the copolymerization of VP with AA at \([M_1+M_2]=30\) mass% and \([M_2]=35\) mol % and in the copolymerization of VP with AAm at the same concentration of the initial monomer mixture they are formed at \([M_2]=15\) and 50 mol %.

In the investigated cases of radiation-induced copolymerization of \(N\)-vinyl-2-pyrrolidone with allyl alcohol and allylamine in sition and in bulk at a dose not exceeding 200 kGy and at 320 K, chain cross-linking
Table 2. Molecular weight characteristics of VP–AA and VP–AAm copolymers obtained by radiation-induced copolymerization in alcohol at D=50 kGy.

<table>
<thead>
<tr>
<th>M₂</th>
<th>Irradiation condition</th>
<th>Copolymers properties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[M₁+M₂] mass (%)</td>
<td>[M₂] mass (%)</td>
</tr>
<tr>
<td>Allyl alcohol</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Initiator (AIBN)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Allylamine</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>50</td>
</tr>
</tbody>
</table>

a) [AIBN] = 1 mass (%) and [M₁+M₂]=3 mass (%).

was not observed. All copolymers well soluble in water. This, in particular, makes it possible to use them as carriers of biologically active compounds.

In the final stage of this work the acute toxicity of copolymer 1₄ ([m₂]=23.2 mol %) and copolymer 2₄ ([m₂]=24.3 mol %) in experiments with mice was determined. Both copolymers were completely non-toxic for warm-blooded animals. The maximum tolerance dose was 6.0 J/kg for copolymer 1₄ and 2.5 J/kg for copolymer 2₄.

Consequently, as a result of these investigations, such conditions of radiation-chemical synthesis of copolymers of N-vinyl-2-pyrrolidone with allyl alcohol and allyl amine were found which make it possible to obtain these copolymers with characteristics (molecular weight, MWD, and toxicity) necessary for using them as modifiers of the properties of various BAC, mainly pharmaceutical products.

We used copolymers of N-vinyl-2-pyrrolidone with allylamine as carriers for the synthesis of salt and amide derivatives of an antiseptic, merthiolate (ethylmercury thiosalicylic acid), effective agents for rendering textile materials stable to microorganisms. On the basis of a copolymer VP with allyl alcohol, a polymer ester of merthiolate was obtained. It exhibits a high level and broad spectrum of fungicidal activity and is of interest as a biocide.

CONCLUSION

Investigation of solution radiation copolymerization process of VP with difficult polymerizable comonomers such as: allyl alcohol and allyl amine has shown that the production of water-soluble non-toxic copolymers VP-AA, VP-Am with high chemical efficiency, narrow molecular weight distribution, variable composition and molecular weight is possible by regulation of parameters in this process. Some of obtained reactive copolymers VP are of a great interest as carriers for both low-molecular weight medicinal compounds and bioactive matters of albumine nature.

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