Poly (maleic anhydride - alt-vinyl acetate) conjugate with Alkylating Agents

I. Synthesis and physico-chemical characterization

The three new polyanionic polymer conjugates were synthesized by the chemical bonding of some N-mustard derivatives of di-(β-chloroethyl)-amine and tri-(β-chloroethyl)-amine with poly(maleic anhydride-alt-vinyl acetate). The chemical structure of the compounds obtained was verified by FTIR, H1-NMR and elemental analysis.

Keywords: poly(maleic anhydride-alt-vinyl acetate) conjugates, alkylating agents

The mustard derivatives represent an important category of anticancer drugs [1-7] but the secondary effects induced by them hinder their use in cancer therapy. Consequently, various research teams are involved in the discovery of the new antitumoral drugs based on alkylating agents characterized by a low toxicity and a similar antitumoral activity [8-11]. The attachment of mustard derivatives as pendant groups of a macromolecular chain appears to be a good solution of this problem [12, 13].

Maleic anhydride (MA) is a unique comonomer because it does not homopolymerize but does form specific copolymers. It is unique in that the copolymers formed are in 1:1 ratio and in an alternating sequence [10]. MA copolymers are known to be good carriers for drugs [13] and enzymes [14-20].

The present paper deals with the synthesis and physico-chemical characterization of a new conjugates of poly(MA-alt-VA) with three mustard derivatives.

Experimental part

Materials

Vinyl acetate (VA) and maleic anhydride (MA) were Merck reagents. Tri-(β-chloroethyl)-amine and di-(β-chloroethyl)-amine were synthesized in our laboratory. The solvents, catalysts and other chemicals used in this paper were Sigma reagents and were used as such.

Product characterization:

The chemical verification was carried out using FTIR spectra recorded on a Digital FTIR 2000 (4000-400 cm⁻¹) and H1-NMR spectra recorded on a JEOL-JNM 60-HL (80 MHz) instrument, in deuterated acetone (only for poly[MA-alt-VA] and conjugate I) and by elemental analysis (Perkin Elmer Instrumental 2400 series CHNS/0 Elemental Analyser).

Synthesis of the compounds

a) Poly(MA-alt-VA). In a glass cylindrical reactor provided with stirrer and condenser, benzene (500 mL) and then MA (50g) was introduced under stirring. Temperature was increased stepwise to 80°C and maintained for 30 min till the MA was completely dissolved. In a Berzelius glass container, freshly distilled VA (40 mL for the MA:VA mole ratio 1:1) and benzoyl peroxide (1.5 g) were mixed and the contents poured into the reactor. The reaction mixture was then maintained at 80°C, under refluxing, for 10h. The resulting copolymer precipitated from the reaction medium when the reaction mixture was cooled. The copolymer was collected and dried in a vacuum oven at 40-50°C for 12 h. A white powder was isolated and the molecular weight (100,000 q.mol⁻¹) (by viscometry in acetone at 30°C) was calculated using the Mark-Houwink relation

\[ [\eta] = 9.32 \cdot 10^6 M_0^{0.34} \]

b) Poly(MA-alt-VA) disodium salt. The hydrolysis of poly(MA-alt-VA) was carried out by the dropwise addition of the copolymer to a cold 5% NaOH solution under continuous stirring until complete dissolution was observed. The solution was dialyzed in water until the pH was ≤ 7. The water was removed in a vacuum rotating vaporizer and the product dried under vacuum at 40°C for 24h.


In a flask provided with reflux condenser, 0.5g of poly(MA-alt-VA) disodium salt; 50 mL of anhydrous acetone and 0.56g (0.0029 mole) of freshly prepared tri (β-chloroethyl)-amine were introduced. The mixture was heated to reflux on a water bath for 45-60 min. The precipitate that formed was removed and the filtrate washed with anhydrous ethanol (40mL)-acetone (15 mL) mixture; the remaining solvent was removed under reduced pressure at 50-55°C. After that the remaining residue (≈5mL) was treated with anhydrous ethyl alcohol-petroleum ether (1:1) mixture and the solid formed was collected and washed with more of the solvent.

* email: marlopapach.utasi.ro; Tel: 0232278683 / 22710
mixture. After drying in vacuum at 40-45 °C for 6 h a yellow colored product was obtained in a 52.2% yield.

d) Poly(β-chloroethylmaleamide acid alt-vinyl acetate) copolymer (conjugate II).

In a flask provided with reflux condenser, 1g (5.4 mM) of anhydrous poly(MA-alt-VA), 30 mL anhydrous dioxan and 0.87 g (5.6 mM of di(β-chloroethyl)amine were introduced. After the reaction mixture was heated at 50-60 °C for 1 h results a homogenous solution. The dioxan was removed by distillation under low pressure at 40-50 °C and the residual viscous product was dissolved in 25 mL of anhydrous acetate. Then anhydrous ethylic ether was added and then dried under vacuum at 40 °C for 8 h. A light yellow product was obtained in 50.2% yield.

e) p-Sulfonated anilide sodium salt of poly(MA-alt-VA).

In a reaction vessel provided with stirrer cooling system and a reflux condenser, 9mL of distilled water and 4.85 g of finally dispersed sulfanilic acid were introduced. A solution of 1.5 g sodium carbonate in 1.5 mL water was added dropwise from a dropping funnel, an intense CO₂ evolvement occurred (the temperature was not allowed to exceed 25-30 °C). To the resulted clear solution containing the sodium sulfonilate solution, 3g of poly(MA-alt-VA) in 15 mL acetone were added stepwise with stirring so that the temperature did not exceed 45°C. A white precipitate formed and the stirring was maintained for another 30-45 min. The product was collected by filtration under vacuum washed with cold acetone and dried at 40-45 °C for 6 h to give a 94% yield.

f) Poly(di-(β-chloroethyl)-aminoethyl maleate-alt-VA)p-sulphonated anilide (conjugate III).

This compound was prepared similarly to conjugate I. Sodium p-sulfonated anilide of poly (MA-alt-VA) (3.8g, 0.01 mole) in 60mL of anhydrous acetone were reacted with 2.3g (0.012) mole) of tri-(β-chloroethyl)amine. A solid dark-yellow colored product was obtained in a 93% yield.

The chemical reactions and the physico-chemical characteristics of the synthesized compounds are given in figures 1-7 and table 1.

Results and discussions

The structure of poly(MA-alt-VA) used as support was conformed by FTIR and H¹NMR. The FTIR spectra of MA-VA copolymer have an oscillation bands for C=O (anhydride) at 1732 cm⁻¹ and at 1438 cm⁻¹ (-CH₂), 1377 cm⁻¹ (CH₃), 1244 cm⁻¹ (-C-O- anhydride, -C-O- ester) and 1031 cm⁻¹ (-C-O- ester).

The H¹NMR spectrum of MA-VA copolymer had peaks at 3.25 ppm for the anhydride groups and 6.6 ppm for the acetate groups. In the case of hydrolyzed copolymer, the signal for the acid groups was observed at 5.80 ppm (ungiven spectra).

The experimental data obtained by elemental analysis confirms the theoretical values (table 1). The synthesis of conjugate I is performed in two stages: the hydrolysis in basic medium of the anhydride ring (fig. 1) and the coupling reaction with the tri((β-chloroethyl)amine (fig. 2).

![Fig.1. The synthesis of disodic salt of poly(MA-alt-VA)](image1)

![Fig.2. The chemical reaction for the synthesis of the conjugate I](image2)

**Table 1**

<table>
<thead>
<tr>
<th>compound</th>
<th>Mᵣ𝐨⁺</th>
<th>Elemental Analysis</th>
<th>Det. %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Calc.%</td>
<td>Det.%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>H</td>
</tr>
<tr>
<td>Poly(MA-alt-VA)</td>
<td>75000</td>
<td>47.5</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>98000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conjugate I</td>
<td>70000</td>
<td>44.8</td>
<td>5.3</td>
</tr>
<tr>
<td>Conjugate II</td>
<td>72000</td>
<td>44.1</td>
<td>5.2</td>
</tr>
<tr>
<td>Sodium Salt</td>
<td>**</td>
<td>44.3</td>
<td>3.68</td>
</tr>
<tr>
<td>Conjugate III</td>
<td>68000</td>
<td>44.2</td>
<td>4.10</td>
</tr>
</tbody>
</table>

* Determined by GPC using Varian Instruments equipped with a PL column and tetrahydrofuran as mobile phase.

** Sodium Salt of poly (MA-alt-VA) modified with p-aminobenzensulfonic acid.
The efficiency of the coupling reaction was estimated by the values of Cl and N contents provided by elemental analysis. The lower determined contents of Cl and N (14.2% Cl and 4.02% N) compared with theoretical ones (26.4% Cl and 5.2% N) suggest that only 77% of –COONa groups were coupled with di-(β-chloroethyl)amine.

The FTIR spectrum of the conjugate has the characteristic bands specific both for the macromolecular support and the mustard derivative: 2989-2877 cm\(^{-1}\) (R-CH\(_2\)-R), 2816 cm\(^{-1}\) (-O-CH\(_3\)), 1691 cm\(^{-1}\) (-C=O ester), 1627-1550 cm\(^{-1}\) (-CH-alkyl), 1350-1238 cm\(^{-1}\) (-CH-alkyl), 1209-1174 cm\(^{-1}\) (-CO-ester) and 870-574 cm\(^{-1}\) (-CCl).

The synthesis of conjugate II is based on the opening reaction of the anhydride cycle of poly (MA-alt-VA) with di-(β-chloroethyl)-amine (fig. 3). The chemical structure of the conjugate II was proved by elemental analysis (table 1), FTIR (fig. 4) and \(^1\)H\(^{1}\)NMR (fig. 5).

The FTIR spectrum of conjugate II has the characteristic bands as follows: 3016 cm\(^{-1}\) (-OH carboxylic), 2947 cm\(^{-1}\) (-CH-alkyl), 1720 cm\(^{-1}\) (-C=O ester), 1679-1629 cm\(^{-1}\) (-C=O acid), 1454 cm\(^{-1}\) (-CH,-alkyl), 1440 cm\(^{-1}\) (-OH carboxylic), 1346 cm\(^{-1}\) (-C-N-amide), 1273-1219 cm\(^{-1}\) (-O-ester) and 860-626 cm\(^{-1}\) (-CCI-). The \(^1\)H\(^{1}\)NMR spectrum also confirms the chemical structure on conjugate II (fig. 3) due to the following signals: \(\delta\) (ppm) 2.375 (m, 1H, CH); 3.375 (dd, 1H, CH); 3.875 (m, 2H, CH\(_2\)); 6.5 (m, 1H, CH); 7.1 (t, 4H, 2CH\(_2\)); 7.6 (m, 4H, 2CH\(_2\)).

Sodium salt of poly(MA-alt-VA) modified with p-aminobenzensulfonic acid (fig. 6) represent an intermediate compound involved in the synthesis of conjugate III.

In this case the experimental data provided by elemental analysis suggests that the opening of the MA cycle by sulfanilic acid have an efficiency of 90%. Regarding the chemical structure, the FTIR spectrum of sodium salt (fig. 7) have the characteristic bands of macromolecular support and for new functional groups inserted by p-aminobenzensulfonic acid [3385-2881 cm\(^{-1}\) (Ar-NH-C-),
Fig. 6. The synthesis of sodium salt of poly(MA-alt-VA) modified with p-aminobenzenesulphonic acid

Fig. 7. The FTIR spectrum of the sodium salt of poly(AM-alt-VA) modified with p-aminobenzenesulphonic acid

Fig. 8. The synthesis of the conjugate III

Fig. 9. The FTIR spectrum of the conjugate III

3182 cm⁻¹ (amidic groups), 3064 cm⁻¹ (OH carboxylic), 2922-2881 cm⁻¹ (-CH-alkyl), 1732 cm⁻¹ (-C=O ester), 1613-1600 cm⁻¹ (-C=O carboxylic), 1577-1423 cm⁻¹ (-CH-aromatic), 1377 cm⁻¹ (-C-SO₃⁻), 1263 cm⁻¹ (-S-O-), 1120-1008 cm⁻¹ (-CH-p-aromatic) and 833 cm⁻¹ (-S=O)].
The conjugate III was obtained by a coupling reaction between the intermediary compounds previously obtained (fig. 6) and tri-(β-chloroethyl)amine (fig. 8).

The chemical structure of the synthesized compounds was proved by the elemental analysis and FTIR spectrum (table 1, fig. 9).

The presence of bands at 3415 cm\(^{-1}\) (Ar-NH-C\(-\)), 3289 cm\(^{-1}\) (NH-amide), 3111 cm\(^{-1}\), 3014 cm\(^{-1}\) (-OH carboxylic), 2945 (-O-CH\(_{3}\) ester), 2777 cm\(^{-1}\) (-CH-alkyl), 1751 cm\(^{-1}\) (-C=O ester), 1629 cm\(^{-1}\) (-C=O amide), 1562 cm\(^{-1}\) (-C-NH-), 1384 cm\(^{-1}\) (-S=O), 1182 cm\(^{-1}\) (-SO\(_{2}\)), 821 cm\(^{-1}\) (-C-Cl-) and 692 cm\(^{-1}\) (C-S-) confirms the structure of conjugate III.

Conclusions

The synthesis of three conjugates of poly(MA-alt-VA) with mustard derivatives was proved by elemental analysis, FTIR and H\(^{1}\)NMR. The key factor of the coupling reaction is the opening of the anhydride cycle by the sodium salt of p-aminobenzenesulfonic acid, as well as by the di(β-chloroethyl)-amine.

References


6. SUNEL, V., POPA, M., POPA, A., SOLDEA, C., Cellulose Chemistry and Technology, 34, nr.3, 2000, p.269


13. DUMITRIU, S., POPA, M., Makromolekuleare Chemie, 189, nr. 1, 1988, p.103


Manuscript received: 5.03.2008