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

I. Synthesis and physico - chemical characterization

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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, H^1-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 par*t*

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 ant 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 Digitals FTS 2000 (4000-400 cm^{-1}) as KBr pellets and H^1NMR spectra recorded on a JEOL-JNMC 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 step wise 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 g.mol) (by viscosimetry in acetone at 30°C) was calculated using the Mark-Houwink relation

$$[\eta]=9.32\,.\,10^{-6}\,M^{0.94}$$

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 *p*H was ≤ 7. The water was removed in a vacuum rotating vaporizer and the product dried under vacuum at 40°C for 24h.

c)Poly(di-(β-chloroethyl-aminoethylester)maleate-alt-VA) (conjugate I).

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 remained residue (≈5mL) was treated with anhydrous ethylic alcohol-petroleum ether (1:1) mixture and the solid formed was collected and washed with more of the solvent

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mixture. After drying in vacuum at 40-45 °C for 6h 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 (5.4mM) 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 8h. 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 funned, an intense CO_2 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 6h 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 H^1NMR. The FTIR spectra of MA_VA copolymer have an oscillation bands for C=O (anhydride). At 1732 cm^{-1} and at 1438 cm^{-1} (-CH-), 1377 cm^{-1} (CH_3), 1244 cm^{-1} (-C-O-C- anhydride, -C-O- ester) and 1031 cm^{-1} (-C-O- ester).

The H^1NMR 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).

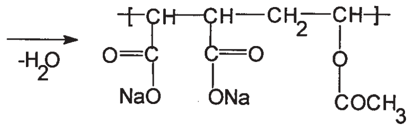
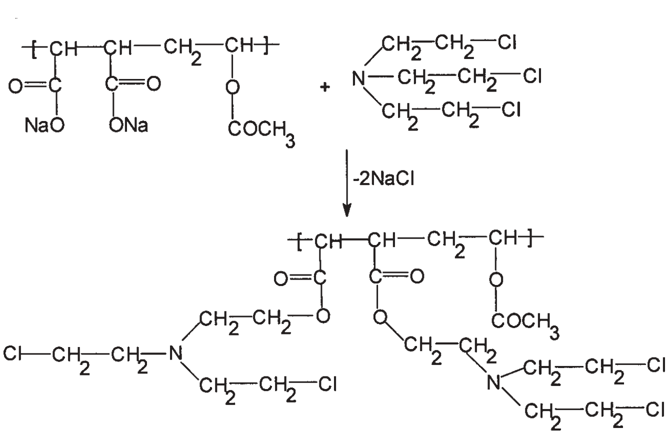


Fig.1. The synthesis of disodic salt of poly(MA-*alt*-VA)



I

Fig.2. The chemical reaction for the synthesis of the conjugate I

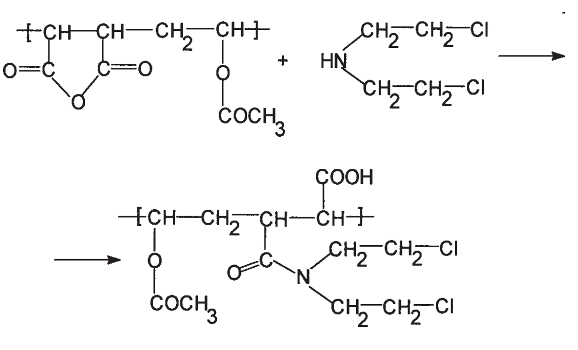
Table 1

THE PHYSICO-CHEMICAL CHARACTERISTICS OF THE SYNTHESIZED COMPOUNDS

compound	**M_n***	**Elemental Analysis**											
		Calc.%						Det.%					
		C	H	O	N	S	Cl	C	H	O	N	S	Cl
Poly(MA-alt-VA)	75000 98000	47.5	5.0	47.5	-	-	-	48.1	4.8	47.2	-	-	-
Conjugate I	70.000	44.8	5.3	18.3	5.2	-	26.4	46.8	6.2	22.6	4.02	-	14.2
Conjugate II	72.000	44.1	5.2	24.6	4.29	-	21.77	44.41	4.32	25.22	5.22	-	20.8
Sodium Salt **		44.3	3.68	33.8	3.68	8.44	6.1 Na%	45.1	3.65	34.5	3.35	7.60	5.8 Na%
Conjugate III	68.000	44.2	4.10	25.2	5.60	6.4	13.90	45.2	5.30	25.7	6.20	5.00	12.60

**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.*



II

Fig.3. The chemical reaction for the synthesis of the conjugate II

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 tri-(β- chloroethyl)amine.

The FTIR spectrum of the conjugate has the characteristic bands specific both for the macromolecular support and the mustard derivative: 2989-2877cm^{-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 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$_2$-alkyl), 1440 cm^{-1} (-OH carboxylic), 1346 cm^{-1} (-C-N-amide), 1273-1219 cm^{-1} (-O- ester) and 860-626 cm^{-1} (-CCl-). The H^{1}NMR spectrum also confirms the chemical structure on conjugate II (fig. 3) due to the following signals: δ (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-),

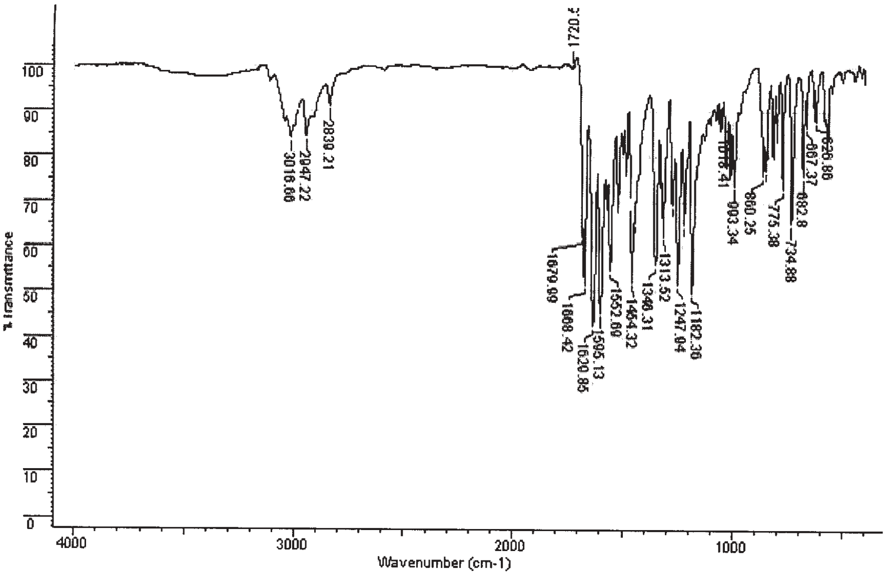


Fig. 4. The FTIR spectrum of the conjugate II

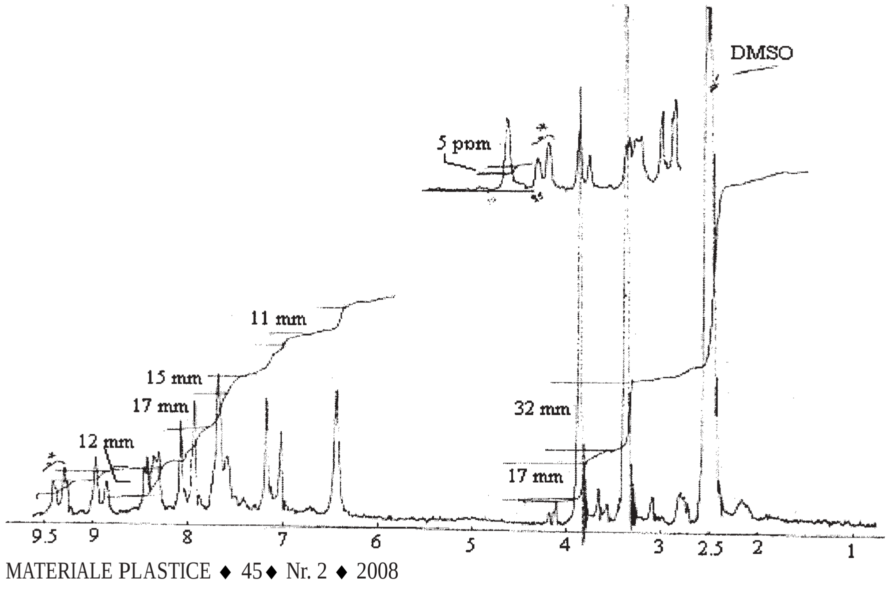


Fig. 5. The H^{1}NMR spectrum of the conjugate II

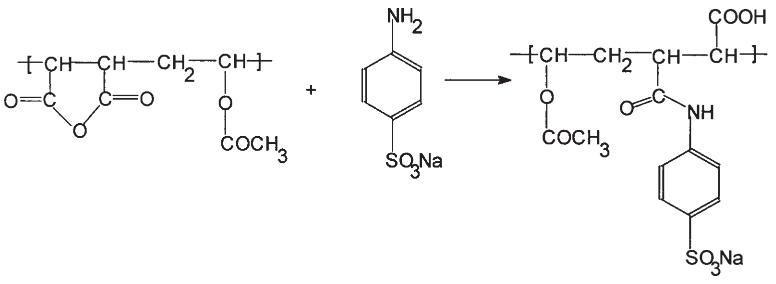


Fig.6.The synthesis of sodium salt of poly(MA-*alt*-VA) modified with p-aminobenzenesulphonic acid

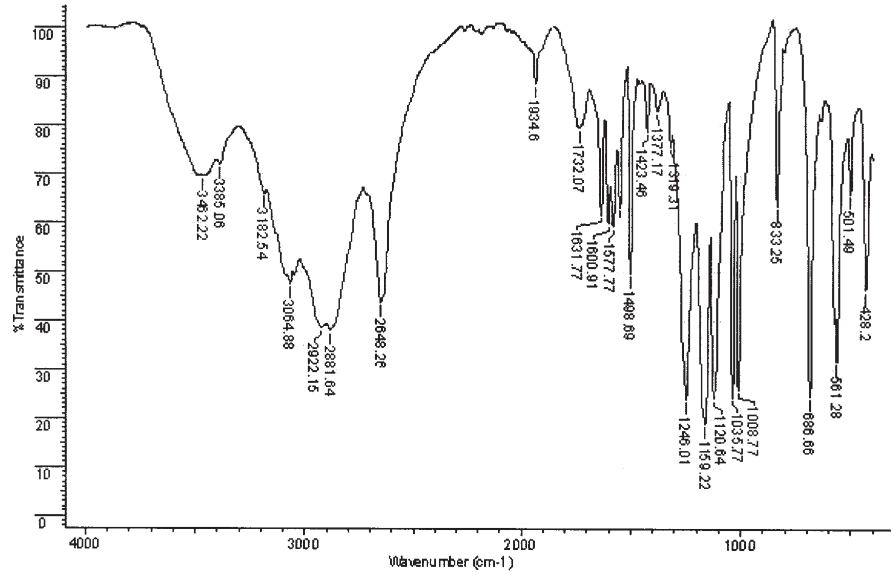
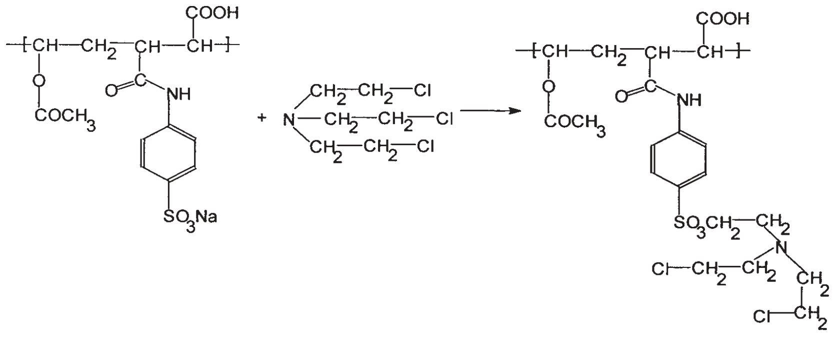


Fig. 7. The FTIR spectrum of the sodium salt of poly(AM-*alt*-VA)modified with p-aminobenzenesulphonic acid



III

Fig. 8. The synthesis of the conjugate III

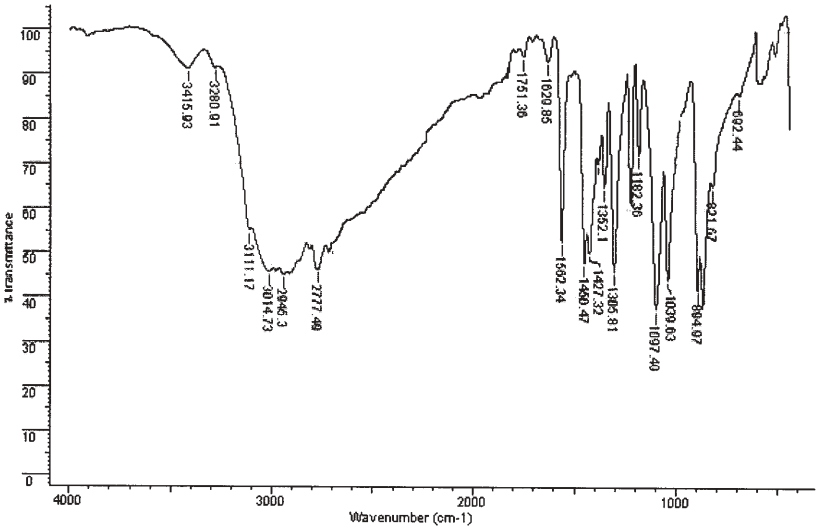


Fig. 9. The FTIR spectrum of the conjugate III

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-SO$_3$-), 1263 cm^{-1} (-S-O-), 1120-1008 cm^{-1} (-CH-p-aromatic)and 833 [redacted] (-S=O)].

The conjugate III was obtained by a coupling reaction between the intermediary compounds previously obtained (fig. 6) and tri-(β-cloroethyl)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

1.UGLEA, C.V., PANAITESCU, L., SPIRIDON, D., URSU, D., POPA, I., OTTENBRITE, R. M., J. Biomater.Sci. Polymer Edn., nr.4, 1996, p.269

2.UGLEA,C.V., OTTENBRITE, R.M., Polysaccharides in Medicinal Applications, Marcel Dekker Inc., Dumitriu, S. Ed, New York, 1997, p.765;

3.UGLEA, C.V., PARV, A., CORJAN, M., DUMITRIU, A., OTTENBRITE, R.M., Rev. Chim., (Bucuresti), **57**, nr.1, 2006, p.16

4.UGLEA, C.V., CORJAN, M., PARV, A., OTTENBRITE, R.M., Rev. Chim., (Bucuresti), **56**, nr.10, 2005, p.1019

5.SUNEL, V., SOLDEA, C., Indian J. Chem., **34(B)**, 1995, p.227

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

7.XIE, G., GUPTA, R., LOWN, J.W., Anticancer Drug Design, **10**, nr.1, 1995, 389

8. GUPTA, R., WANG, H., HUANG, L., LOWN, J. W., Anticancer Drug Design, **10**, nr.1, 1995, p.25;

9.SUNEL, V., SOLDEA, C., Comp Rend. Acad. Bulg. Sci., **46**,nr.1,1993,p.61

10.SUNEL, V., CECAL, A., SOLDEA, C., ASANDEI, N., Rev.Roumaine de chimie, **40**, nr.5, 1995, p.763

11.JANSON , P.E., KENNE, L., LINBERG, B., Carbohydr, Res., **45**nr.2, 1975, p. 275

12.POPA, M., SUNEL,V., BALAITA-RUSU, L., Journal of Biomaterials Applications, **18,** nr.1, 2003, p.83

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

14.PATEL, H., RAVAL, D. A., MADAMWAR, D., PATEL, S.R., Angew. Makromol. Chem., **245**, nr.1, 1997, p.1

15.PATEL, H., RAVAL, D. A., MADAMWAR, D., PATEL, S. P., Angew. Makromol. Chem., **263,** nr.1, 1998, p.25

16.LEE, Y. S., BYOUN, Y. S., Bull. Korean Chem. Soc., **23**, nr. 11, 2002, p. 1833

17.JEONG, J. H., BYOUM, Y.S., LEE, Y. S., Reactive and Functional Polymers, **50**, nr.3, 2002, p. 257

18.UGLEA, C. V., PARV, A., DUMITRIU, A., CORJAN, M., OTTENBRITE, R., J. Bioact. Compat. Polym., **21,** nr.5, 2006, p. 399

19.SUNEL, V., POPA, M., DUMITRIU, C. L., CIOBANU, A., BUNIA, I., POPA, A. A., Reactive and functional polymers, **65**, nr.3, 2005, p. 367

20.BACU, E., CHITANU, G. C., COUTURE, A., GRANDCLAUDON, P., SINGUREL GH., CAPROV, A., European Polymer Journal, **38**, nr. 11, 2002, p. 1509

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