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Biodegradable poly(vinyl alcohol-lactic acid-aspartic acid) copolymers

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Abstract: The aliphatic polyesters: polylactides (PLA), polyglycolides (PGA), poly(ε-caprolactone) (PCL) as well as their copolymers especially, poly(lactide-co-glycolide) (PLGA), poly(vinyl alcohol) (PVA), chitosan, dextran and starch occupy first place among biodegradable, biocompatible and non-toxic polymers. PVA, with different molecular weights, hydrolysis degree, tacticity, crystallinity, and due to the abundance of pendant hydroxy groups in the backbone is capable of several grafting and cross-linking. The present study concerns the synthesis of graft copolymers poly(vinyl alcohol) with lactic acid (LA) and aspartic acid (Asp). The modification reaction of poly(vinyl alcohol) with lactic and aspartic acid was achieved by solution polycondensation using dioxane/water (3/1) as solvent mixture, different molar ratios PVA/LA/Asp and manganese acetate as catalyst: $Mn(CH_3COO)_2$ $4H_2O$. The copolymers were characterized by FTIR, ^{1}H-NMR, thermal stability (DSC, TG), elemental analysis, wide angle X-ray diffraction (WAXD). The average particle size and zeta potential for aqueous dispersions of copolymers, solubility in organic solvents and biodegradation testing on agar-agar medium inoculated with a fungus *Chaetomium globosum* class MO5 stock, were studied.

Introduction

Biodegradable polymers are designed to degrade upon disposal by the action of living organisms. Extraordinary progress has been made in the development of practical processes and products from polymers such as: starch, cellulose, lactic acid, glycolic acid and their copolymers, poly(vinyl alcohol) ,chitosan, and collagen. The need to create alternative biodegradable water-soluble polymers for down-the-drain products such as detergents and cosmetics, hydrogels, matrices for medical applications has taken on growing importance.

Poly(vinyl alcohol), a hydrolysis product of polyvinyl acetate, is well suited for blends with natural or synthetic polymeric materials since it is highly polar, can be manipulated in water solutions, and is also biodegradable and biocompatible [1]. Conventional PVA cannot be processed by traditional technologies since it decomposes close to its melting point of 230 ^{0}C. PVA films and molded items have good physical properties such as tensile strength and elongation at break. PVA blends with starch [2-5], chitosan and pectin [6-8] have been prepared by casting of water solutions and subsequently proposed on the market for use as agricultural mulch films and as water soluble laundry bags. Chitosan membranes blended with PVA have already been reported to have good mechanical properties for medical products, controlled delivery of drugs, and for food applications [9, 10]. Blends of PVA with natural or synthetic polymers need components compatibility or physical or chemical linkages in the reactive blending, also both components must be soluble in common solvents in the case of films from solutions. Mostly, these conditions are difficult to accomplish, for this reason PVA grafting reactions are necessary.

Polylactic acid is a linear aliphatic polyester produced by polycondensation of lactic acid or by the catalytic ring opening polymerization of the lactide. The ester linkages in PLA in Poly(DL-lactic–co-glycolic acid) (PLGA) are sensitive to both chemical hydrolysis and enzymatic chain cleavage. Poly(DL-lactic–co-glycolic acid) is one of the most widely used biodegradable polymers since it is biocompatible and its degradation rate and mechanical properties can be easily controlled by varying the copolymer ratio of lactic acid to glycolic acid [11]. As aliphatic polyesters are biodegradable and biocompatible, presenting high flexibility and good tensile strength [12, 13], they can be used to achieve some PVA blends or copolymers that can be used not only in medical materials, but also processed in films, molded products, laminates, foams, non-woven material, and coatings [14, 15]. Porous PLGA/PVA scaffolds were fabricated by blending PLGA with PVA to improve the hydrophilicity and cell compatibility of the materials for tissue engineering applications [16]. PLGA/PVA blend with different PVA compositions up to 20 wt.% were fabricated by a melt molding particulate leaching method (non-solvent method).

New polymeric materials are constantly sought for food packaging applications. PVA is a water soluble synthetic polymer with excellent film-foaming, emulsifying, and adhesive properties. This versatile polymer offers outstanding rersistance to oil, grease and solvents, high tensile strength, flexibility, and high oxygen and aroma barrier [17]. However, straight PVA is not used in food packaging for that PVA film is difficult to extrudate. Therefore, PVA blending with different polymers or graft copolymerization are versatile methods for providing functionality to the resulting polymers, and for regulating biodegradable and biocompatible properties.

PVA, with a raised structural variability (different molecular weights, hydrolysis degree, tacticity, crystallinity, particle size distribution), and due to the abundance of pendant hydroxy groups in the backbone is capable of several grafting and cross-linking reactions with acid chlorides, acid anhydrides, acrylamide, lactic acid or lactic acid oligomer, glycolic acid [18-21].

In this paper we present the study concerning the synthesis and characterization of several biodegradable copolymers obtained by grafting poly(vinyl alcohol) with lactic acid and aspartic acid, by solution polycondensation procedure using dioxane/water (3/1) as solvent mixture, different molar ratios PVA/LA/Asp, and manganese acetate as catalyst. Films obtained from some copolymers were exposed to biodegradation with fungus *Chaetomium globosum.*

Results and discussion

FTIR spectra of unmodified PVA and a representative PVA-LA-Asp copolymer are presented in Figure 1. In the case of PVA-LA-Asp copolymer and PVA spectra the presence of some wide absorption bands at 3423 cm^{-1} and 3434 cm^{-1} respectively, are observed. These peaks can be assigned to ν_{OH} of the stretching vibration modes of the hydroxyl groups or water of crystallization in PVA and of the OH unreacted groups, as well as to ν_{NH2} vibration of the alkyl-NH_2 group in the copolymer. The ester group at 1733 cm^{-1} (ν_{CO}) present in the copolymer is obtained by the reaction of hydroxyl groups from PVA with the carboxyl groups present in lactic and aspartic acid. At 1593 cm^{-1} we remark the presence of a peak corresponding to COO^{-}

stretching vibrations corresponding to unreacted carboxyl groups of the aminoacid. Also, we remark the presence of some symmetrical and asymmetrical (ν_{CO}) ester bands present in the copolymer between 1044-1249 cm^{-1}. The absorption bands present in the copolymer between 1320-1424 cm^{-1} (ν_{CO} and δ_{OH}) can be attributed to the unreacted carboxyl groups of aspartic acid. Also, in the copolymer we can notice the presence of a high intensity absorption band at 1599 cm^{-1} and of a shoulder at 1641 cm^{-1} (δ_{NH} vibrations of I-aminoacid band) and at 1453 cm^{-1} and 1598 cm^{-1} (δ_{NH} vibrations of II-aminoacid band), resulting from aspartic acid grafted in the copolymer structure. The presence of PLA homopolymer in the synthesized products is excluded, as PLA is soluble in acetone and ias removed by extraction. These characteristic absorption bands noticed in the copolymers provide substantial evidence of PVA grafting reaction.

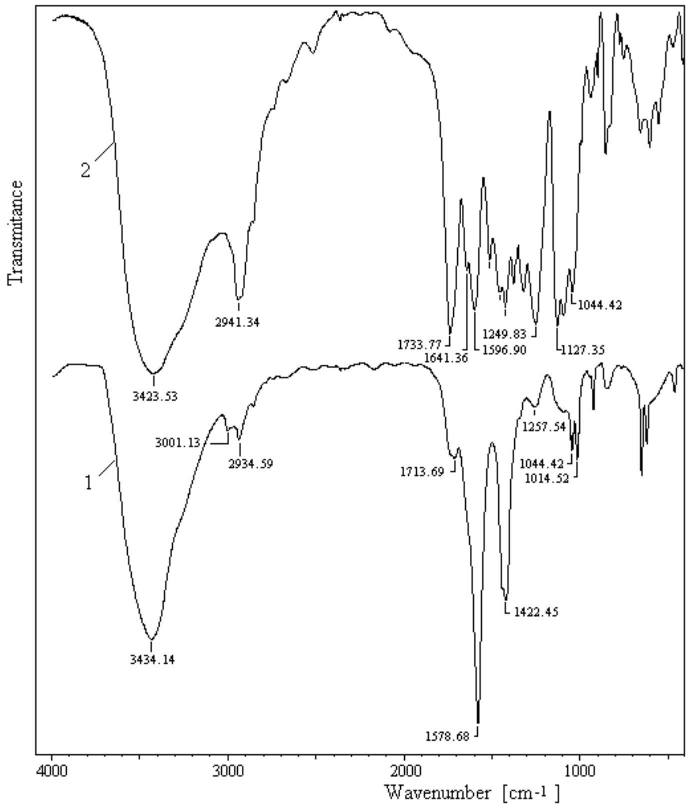

Fig. 1. FTIR spectra: 1- PVA; 2- PVA-LA-Asp copolymer.

^{1}H-NMR spectrum of PVA-LA-Asp copolymer and the possible structure are presented in Figure 2. Based on the reports of peak assignments for proton species in PVA, PLA, polysuccinimide (PSI) and their derivatives [22-27], we assigned the proton signals in the ^{1}H-NMR spectrum of PVA-LA-Asp copolymer, as given in Figure 2. Residual protons signal in D_2O were set at 4.785 ppm. The resonance at δ=1.672 ppm was attributed to the protons in CH_2 groups (a-a') of the PVA backbone, at δ=3.745 ppm and δ=3.930 ppm to the protons in methine groups of PVA structure (b-b'). In the region 4.316-4.429 ppm, grouped peak assignements were noticed, that were attributed to the protons of the CH groups in PLA and polyaspartic acid structure (c, c', c'') slightly displaced due to the influence of the atoms in the immediate neighbourhood.

In these reaction conditions, possibly polysuccinimide was also obtained as an intermediary by-product, then the succinimide cycle could be broken-up and two structural units α- and β-type could be constituted [20, 21]. The proton signals of the CH_2 groups from α and β structural units of aspartic acid (d and e protons) are located at 2.887 ppm and 2.650 ppm respectively, the shift is influenced by the nature of the near atoms. The protons of CH_3 group (f) from lactic acid structural unit are localized at 1.285 ppm, and the protons due to NH_2 group (g) at 2.000-2.085 ppm.

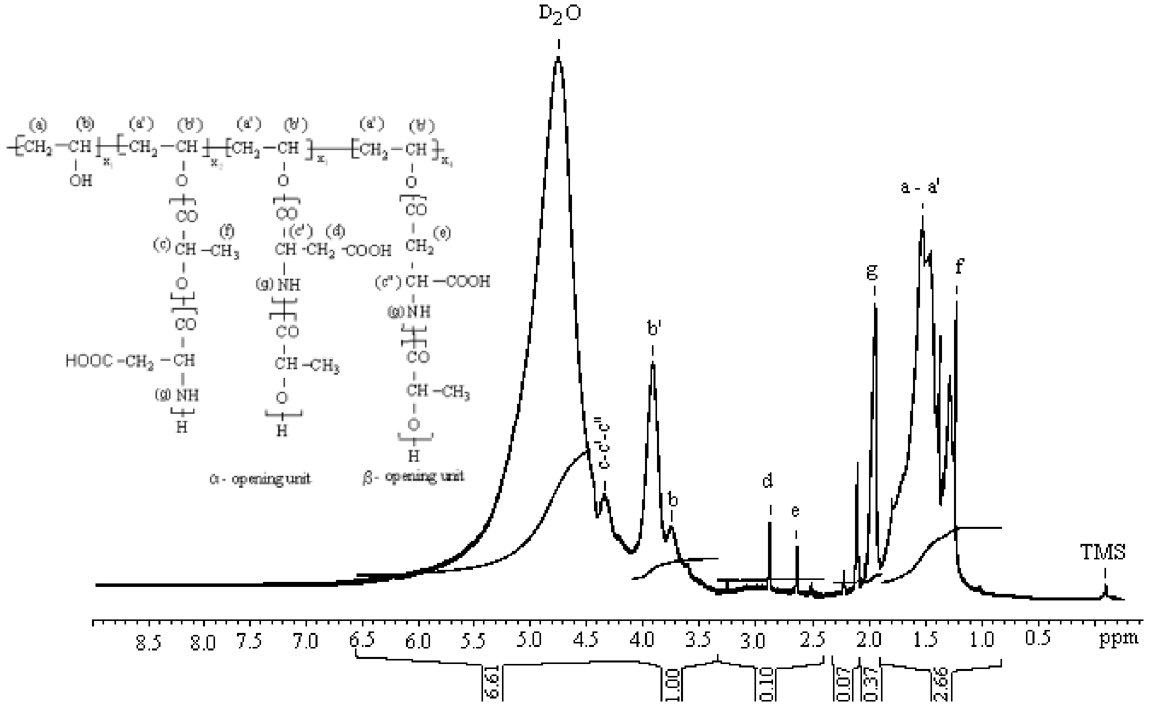

Fig. 2. ^{1}H-NMR spectrum of PVA-LA-Asp copolymer.

The thermal behavior of PVA and copolymers is analysed by thermogravimetry (TG), and some data are presented in Figures 3, 4 and Table 1. The thermogravimetric curves present a main process of thermal decomposition situated on Ti-Tf temperature interval where the weight losses take place with the highest speed, and other 3 secondary processes where these losses take place with a more reduced speed. PVA thermal stability is more raised comparative with the synthesized copolymers. Weight losses of 10 wt.% are recorded in the case of PVA at 280 ^{0}C, and in the case of copolymers these losses are observed on temperature interval 165-205 ^{0}C and vary with PVA/LA/Asp molar ratio in the initial mixture of reaction. Weight losses of 50 wt.% in the case of PVA are recorded at 335 ^{0}C, and in the case of copolymers at 305-327 ^{0}C. The copolymers A, B, C that contain a higher content of aspartic acid in the synthesis (0.075 moles) present a better thermal stability than D, E, F ones, these results suggest that aspartic acid (trifunctional monomer) can lead to a more advanced grafting degree in copolymers during synthesis. The activation energy (Ea) and reaction order (n) were determined by Coats-Redfern method [28-30] on T_i-T_f temperature interval.

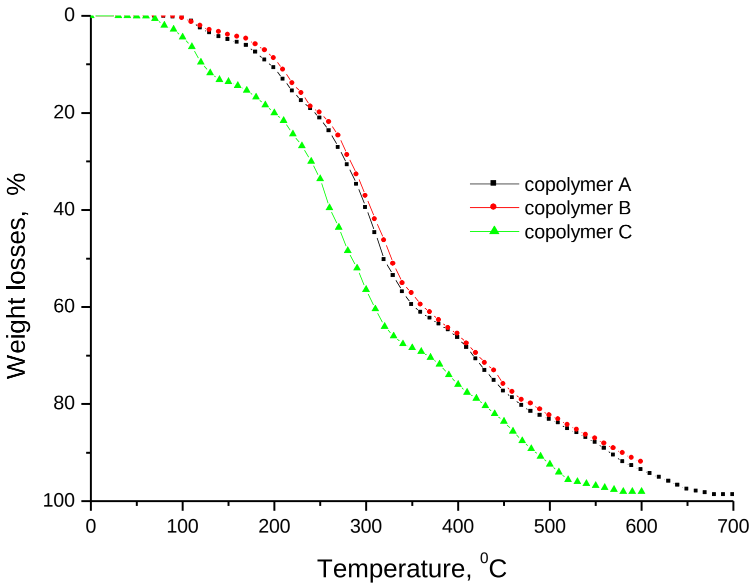

Fig. 3. TG diagrams of PVA-LA-Asp copolymers.

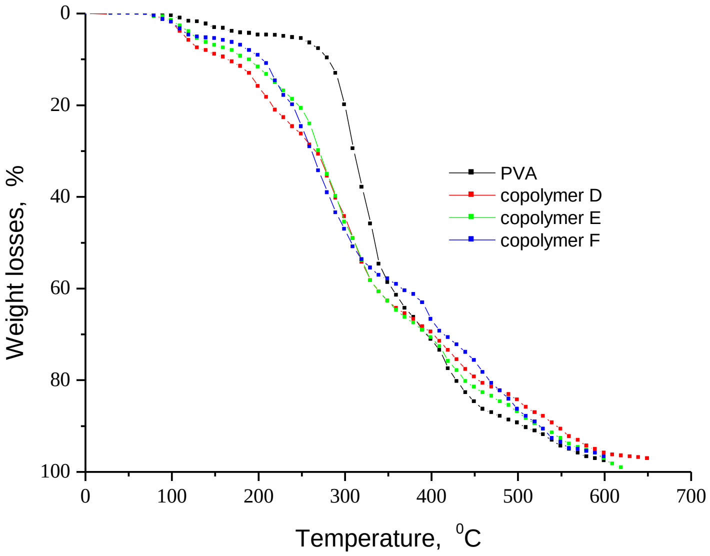

Fig. 4. TG diagrams of PVA and PVA-LA-Asp copolymers.

The activation energy as a function of the conversion degree of the thermal decomposition processes (Fig. 5), presents a quick lowering to a conversion degree of 0.05 (PVA) and 0.15-0.2 (PVA-LA-Asp copolymers). At a conversion degree higher than 0.2, the variation of the Ea lowers at a much reduced rate, while in the case of PVA we can notice a slight growth. The sudden lowering of Ea observed in the first part of the interval, suggests that at the beginning the decomposition reaction has an autocatalytic behaviour, due to oxygen traces in the copolymers that act as catalyst of the thermal decomposition processes.

Tab. 1. Thermal characteristics determined by thermogravimetry.

Sample	T_{10} ^{0}C	T_{50} ^{0}C	T_i ^{0}C	T_m ^{0}C	T_f ^{0}C	W_{Ti-Tf} %	W_{600} %	E_a kJ/mol	n
PVA	280	335	225	312	385	63	97.6	146.15	2.0
A	198	320	232	302	380	46	95.5	106.78	1.6
B	205	327	240	315	380	44	94	124.97	1.8
C	205	310	210	270	358	48	98	85.74	1.4
D	165	310	237	295	387	44	97.2	59.50	1.3
E	185	310	240	295	387	50	98.5	78.39	1.7
F	200	305	135	262	365	54	96.8	100.95	3.0

T_{10}, T_{50} - temperature corresponding to 10 % and 50 % weight loss.
$T_{i,}$ $T_{f,}$ - the initial and ultimate temperature of the domain on which Ea and n were calculated.
T_m – temperature corresponding to weight losses with maximum rate.
W – weight losses.
Ea, n – activation energy and reaction order determined on the main thermal decomposition process (T_i-T_f).

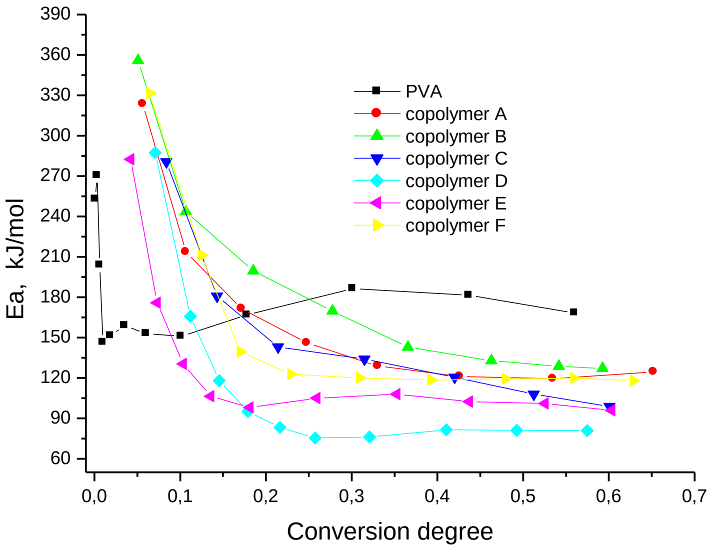

Fig. 5. Variation of Ea on conversion degree during thermal decomposition reaction.

In Figures 6, 7 and Table 2 thermal characteristics of PVA and the copolymers determined by differential scanning calorimetry (DSC) are presented. The glass transition temperatures (T_g) of the copolymers have lower values when compared to PVA. The glass transition temperatures grows with lactic acid growth ratio in the initial reaction mixture, and the raise of aspartic acid from 0.05 to 0.075 mol determines a slight lowering of the T_g value. The glass transition temperatures of A, B, and C copolymers present lower values comparative to the T_g of the copolymers D, E, F (Table 2). The values of the specific thermal capacities (ΔC_p) of the copolymers are different for PVA ones (ΔC_p=0.534 J/g·^{0}C), suggesting that the amorphous/crystalline ratio is modified and depend on copolymer composition. The melting temperatures (T_m) of the copolymers were generally more raised, it was ascertained a variation of the values in the temperature interval comprised between 148-204 ^{0}C was a function of the molar ratio of the components in the initial reaction mixture of respective copolymer, comparative to 139-188 ^{0}C in the case of PVA. The melting enthalpies (ΔH) of the copolymers record some modifications comparative to PVA (ΔH=63.777 J/g), the modifications being also influenced in this case by the ratio of the three components in the composition. The copolymers with molar ratio PVA/LA 1/1 (copolymers A and D) present a lower melting enthalpy comparative to copolymers with the molar ratio 2/1 (copolymers B and E), its value is more close in this case with the melting enthalpy of PVA. In the case of the copolymers with molar ratio PVA/LA 1/2 (copolymers C and F), Tm and implicit ΔH were not emphasized until 235 ^{0}C these copolymers presented also the highest T_g values, approximately 42 ^{0}C.

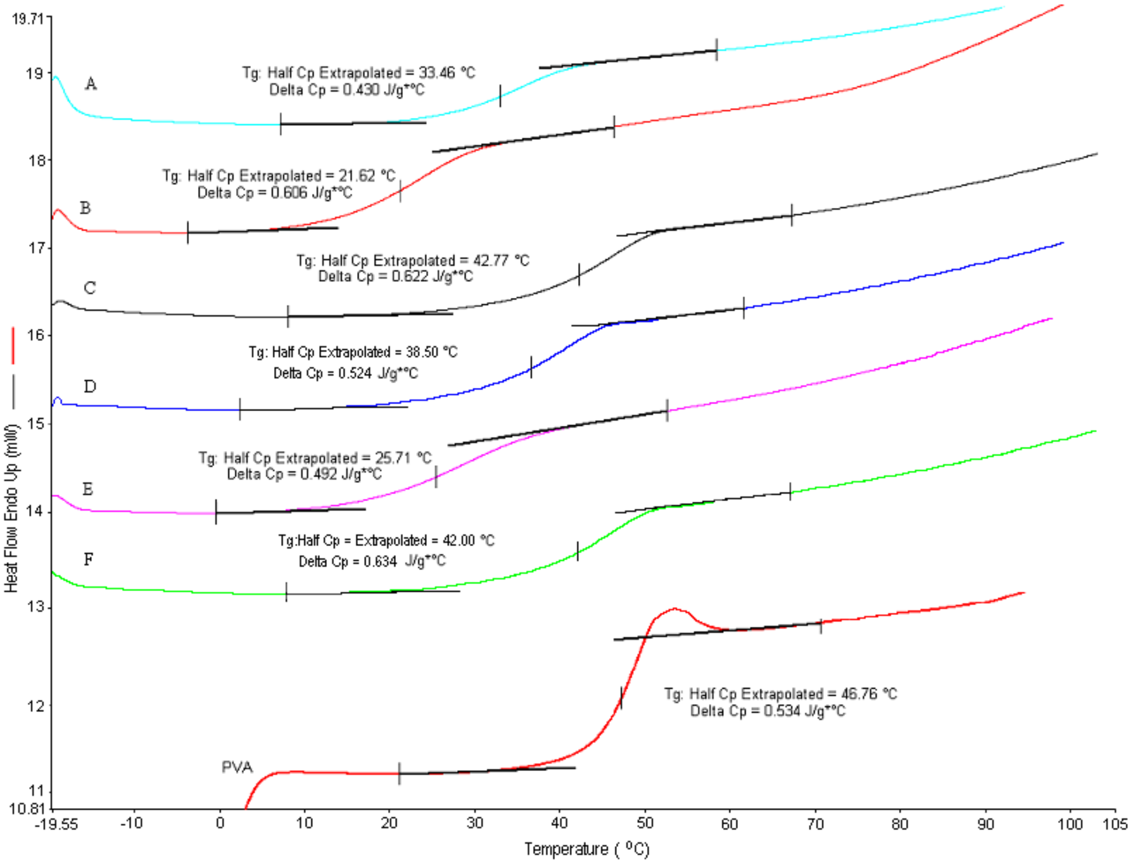

Fig. 6. DSC curves: glass transition temperatures of PVA and PVA-LA-Asp copolymers.

Tab. 2. Thermal characteristics determined by DSC.

Sample	Tg ^{0}C	ΔCp J/g · ^{0}C	Tm ^{0}C	ΔH J/g
PVA	46.76	0.534	139-188	63.776
A	33.46	0.430	152-200	54.014
B	21.62	0.606	159-202	75.056
C	42.72	0.622	-	-
D	38.50	0.524	148-198	52.234
E	25.71	0.492	152-204	56.318
F	42.00	0.634	-	-

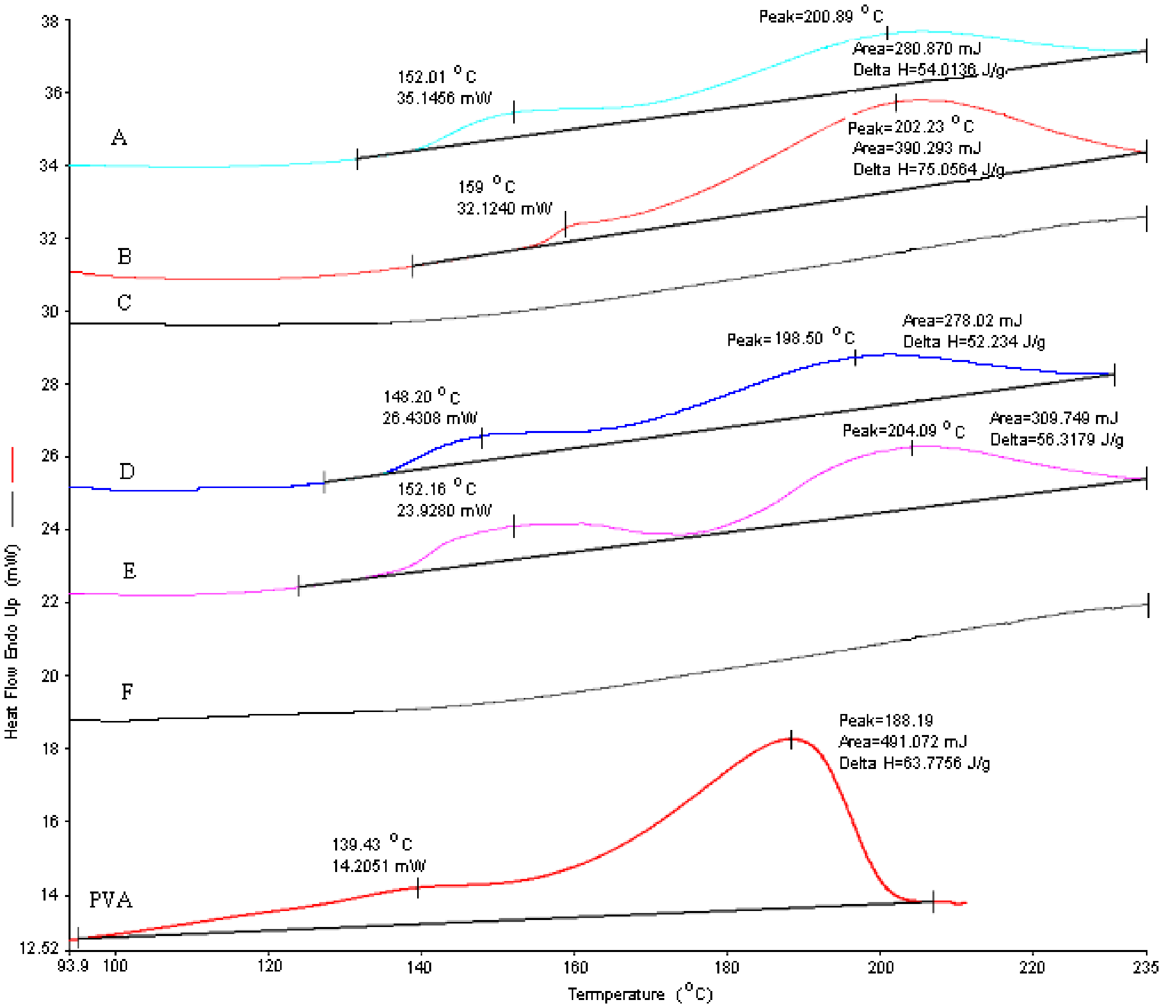

Fig. 7. DSC curves: melting temperatures of PVA and PVA-LA-Asp copolymers.

The solubilities of graft copolymers PVA-LA-Asp in different organic solvents comparative to PVA were studied at room temperature and 60 ^{0}C (Table 3). Generally, from data presented in table we can notice that the copolymers present better solubilities in water, DMF, DMSO, methanol solvents with raised solubility parameter.

Tab. 3. Copolymers solubilities in organic solvents at room temperature and at 60 ^{0}C.

Sample	Solvent/Solubility parameter (cal/cm^3)$^{1/2}$						
	Water/ 23.4	DMF/ 12.1	DMSO/ 14.5	Methanol/ 14.5	Ethanol/ 12.92	Ethyleneglycol/ 14.6	Dioxane/ 7.9
A	+/+	-/+	+/+	0/-	0/-	0/-	0/-
B	+/+	-/+	+/+	-/+	0/-	0/+	0/-
C	-/+	-/-	-/+	-/+	0/-	0/-	-/-
D	+/+	-/+	-/+	0/0	0/-	0/-	0/0
E	+/+	-/+	+/+	-/+	0/0	-/+	0/0
F	+/+	-/+	+/+	-/+	+/+	-/-	-/-
PVA	+/+	0/0	+/+	-/-	0/0	0/-	0/0

+ soluble; - slightly soluble; 0 insoluble.

Also, the copolymers solubility was greatly influenced by temperature, comparative to PVA suggesting that the structure and properties are changed. Thus, the solubility improvement in the utilized organic solvents could be influenced by the appearance of some ester linkages in the copolymers structure.

The elemental analysis of the synthesized copolymers (Table 4) show that nitrogen exists in the copolymers structure (from aspartic acid) and content varies between 1.69-4.17 wt.%, and depends on the initial molar ratio of the components in the grafting reaction. Nitrogen presence in the copolymers structure confirms that the grafting reaction was carried out and encourages certain statements.

Tab. 4. The elemental analysis of copolymers.

Sample	C wt.%	H wt.%	N wt.%
A	53.12	5.55	2.32
B	58.26	5.19	2.44
C	54.87	3.76	4.17
D	59.01	6.08	1.69
E	51.32	5.37	2.12
F	63.33	5.22	3.86

In Table 5 are presented the dimensional characteristics (Z-average size), zeta potential and electrical conductivity of the copolymer aqueous dispersions (0.01 g/L). The copolymers were dispersed in bidistilled water, and data were recorded at 25 ^{0}C. From data presented in table, we can notice that the average size of the synthesized copolymers is lower than that of PVA. This fact can be due to a higher packing degree of grafted copolymers comparative to PVA. The packing degree is also influenced by the hydrogen linkages that can be formed between some unmodified carboxyl groups that belong to grafted aspartic acid units, as well as hydroxyl groups unmodified from PVA structure. Also, zeta potential presents lower absolute values in the case of copolymers suggesting a more reduced negative electrical charging (except copolymer F with zeta potential value -10.3 mV), and the electrical conductivity presents more raised values in the case of the copolymers when compared with PVA.

Tab. 5. Characteristics of the copolymer dispersions determined at 25 ^{0}C.

Sample	Z-average size nm	Polydispersity index	Zeta potential		Conductivity mS/cm
			Z.P. mV	pH	
PVA	247	0,405	-5.69	5.28	0.333
A	205	0,568	-1.98	4.02	1.34
B	195	0,734	-2.51	3.77	1.20
C	242	0,598	-5.69	4.33	1.36
D	173	0,556	-2.49	4.58	1.46
E	69.8	0,973	-3.30	4.43	1.45
F	79.9	1,000	-10.3	4.14	0.645

The electrical conductivity and zeta potential was studied in 25-40 ^{0}C temperature interval. In all cases a linear growth of the electrical conductivity with temperature is remarked (Fig. 8). The lowest values of the electrical conductivity are observed in the case of PVA and of copolymer F, and zeta potential values are differently influenced by temperature (Fig. 9). Zeta potential refers to electrostatic potential generated by ions accumulation at the surface of particle (or colloid) organized in a double electric layer built up of Stern layer and a diffusion layer. Zeta potential was measured at pH values (presented in Table 5), and its values were situated inside the interval ±30mV. In general, it is known that zeta potential of stable colloidal solutions has values situated outside ±30 mV domain. Since the copolymer dispersions achieved have lower absolute values of zeta potential than those earlier mentioned, they would not present time stability and the tendency of sedimentation is possible.

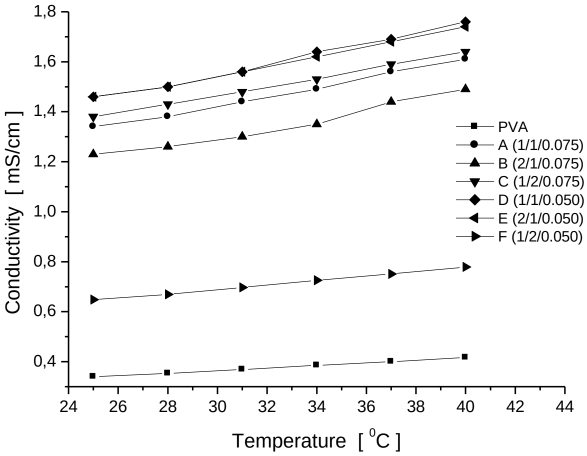

Fig. 8. Conductivity versus temperature.

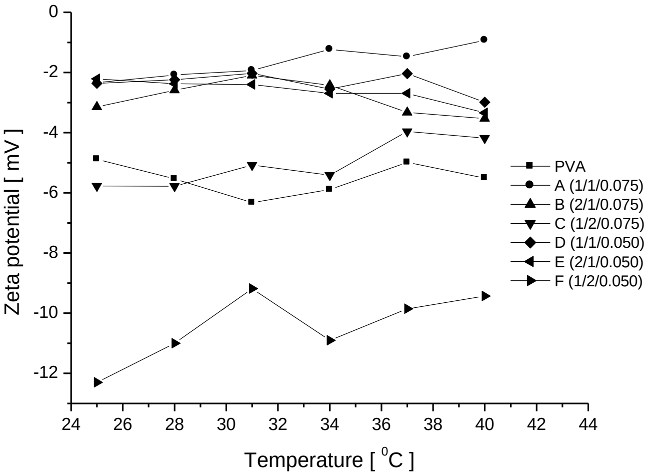

Fig. 9. Zeta potential versus temperature.

Figure 10 presents the wide angle X-ray diffractograms of PVA and PVA-LA-Asp copolymers, and in Table 6 are given comparatively the peak intensity values at the same values of 2θ angle. The appearance of new peaks in copolymers X-ray diffractograms and the slight shift of diffraction angles of characteristic peaks can be due either to a co-crystallization process, or to the modifications of the base cell parameters in the copolymers structure. PVA diffractogram presents a broad peak of high intensity (115. 40 a.u) at about $2\theta=20^0$, comparable to the one presented in literature [31, 32], indicating its crystalline structure. The breadth of the peak at $2\theta=20^0$ and the lack of other main peaks on 2θ interval between $5\text{-}60^0$, suggests that PVA presents a reduced degree of crystallinity. In the case of copolymers, the peaks intensities recorded at the same value of 2θ angle (20^0) are much lower (approximately 63 a.u.) in the case of the copolymers with 0.05 mol aspartic acid in the reaction mixture (copolymers D and F) and 75 a.u. (copolymer A with 0.075 mol aspartic acid). Generally, the crystallinity trend of PVA-LA-Asp copolymers follows the PVA diffractogram, but some changes concerning the intensities of the peaks at other values of 2θ angle values were observed. Namely, in the 2θ region between 20^0 and 49^0 appear some crystalline reflections of more raised intensities, supposing a modification of crystallinity comparative to PVA. In the case of copolymer D an additional narrow peak is observed, (intensity 29 a.u.) at $2\theta=39.5^0$. Also, by the deconvolution method of WAXD curves the degree of crystallinity, was determined. The method is based on calculating the proportion of the crystalline phases area reported to the total surface area. The degree of crystallinity (α) was determined with the formula:

$$\alpha = (A_1/A_1+A_2) \cdot 100$$

where A_1 – crystalline phase area; A_2 – amorphous phase area.

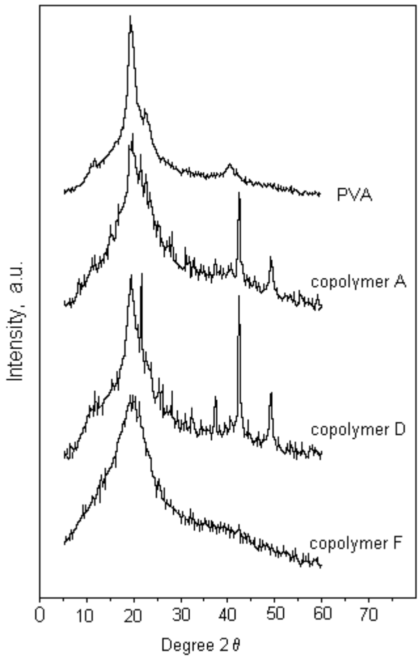

Fig. 10. WAXD patterns for PVA and PVA-LA-Asp copolymers.

The degree of cristallinity of the copolymers undergoes certain modifications and is influenced by the molar ratio of the components in the initial reaction mixture, the obtained values are generally much lesser than PVA ones utilized in the grafting reaction. The modification of PVA was also noticed in the solubility study when an improvment of the copolymers solubility in certain organic solvents was observed (DMF, methanol, etc).

Tab. 6. Peak intensity values and degree of crystallinity.

	Intensity (a.u.)			
2θ	PVA	copolymer A	copolymer D	copolymer F
20	115.4	75	63.2	63
39.5	-	-	29	-
41.5	31.6	39.7	55	29.2
49	17.8	32.6	22	22
α [%]	25.67	13.56	19.20	18.45

Biodegradation testing

To determine copolymers biodegradability, they were tested in controlled laboratory conditions in the presence of fungus inoculated on agar-agar medium. The experiment was carried out using Petri dishes, and the most representative images concerning the biodegradation dynamics (Figs.11, 12), were selected. Temperature was maintained at 28 ^{0}C with a thermostat and the biodegradation monitoring was carried out after 4 and 14 days from the film application. The observations ascertained during the experiment shows that both copolymers were strongly colonized by the fungus micellium *Chaetomium globosum,* demonstrating that these copolymers can be biodegradable.

To establish the biodegradation degree of the copolymers, a biochemical marker such as, catalase from the fungus micelle cultivated in the conditions earlier presented, was determined. To determine the catalase activity, the iodometric method with sodium thiosulphate was utilized [33, 34]. In acid medium, hydrogen peroxide that is not decomposed when the catalase action finishes oxidizes potassium iodide as given in the reaction scheme:

$$2KI + 2H_2O_2 + H_2SO_4 \text{------>} I_2 + 3H_2O + K_2SO_4 + 1/2O_2$$

The iodine resulted was titrated with sodium thiosulphate as given in the reaction:

$$3I_2 + 6Na_2S_2O_3 \text{------>} 6NaI + 3Na_2S_2O_6$$

The catalase activity was expressed in mg H_2O_2 decomposed in the following reaction:

$$H_2O_2 + H_2O_2 \text{----->} 2H_2O + O_2$$

The unit of catalase activity represents the enzyme quantity that decomposes a μmol of H_2O_2 in 5 minutes at 20 ^{0}C. The specific activity is reported with respect to protein:

$$\text{specific activity} = \mu\text{mol } H_2O_2/\text{mg protein}$$

Data concerning the catalase activity in the fungus micelle for the studied copolymers present the next values of this enzyme:copolymer D = 260,3 UC/min/mg, copolymer F = 341,6 UC/min/mg.

In the copolymer with molar ratio PVA/LA (1/2) (copolymer F), the catalase activity is higher meaning that lactic acid from the copolymer structure raises the biodegradation susceptibility.

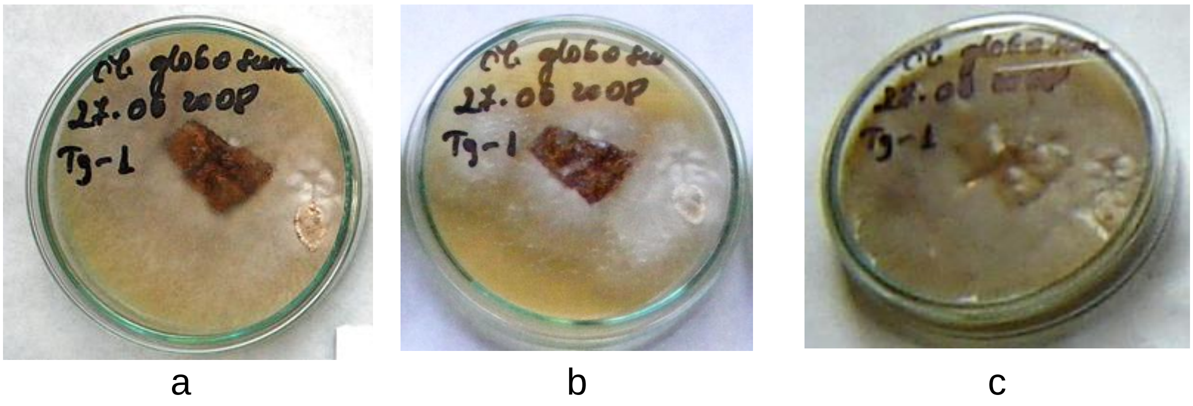

Fig. 11. Experiment concerning copolymer biodegradation (copolymer D): a - culture of *Chaetomium globosum* on which the copolymer was applied seven days after preparation; b - biodegradation dynamics after 4 days; c - biodegradation dynamics after 14 days.

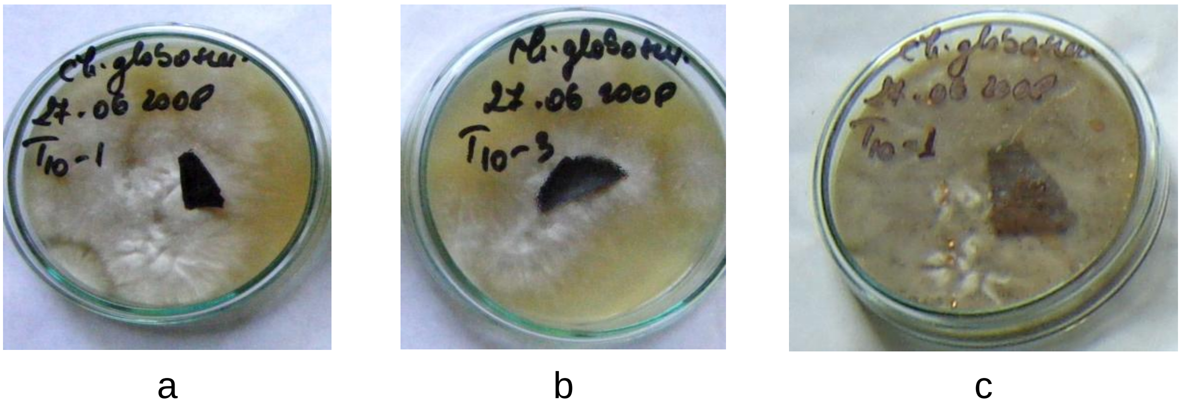

Fig. 12. Experiment concerning copolymer biodegradation (copolymer F): a - culture of *Chaetomium globosum* on which the copolymer was applied seven days after preparation; b - biodegradation dynamics after 4 days; c - biodegradation dynamics after 14 days.

Conclusions

Copolymers based on PVA-LA-Asp have been synthesized. FTIR spectra,^{1}H- NMR and elemental analyses demonstrate that lactic acid and aspartic acid have been grafted on PVA. DSC and TG analyses prove that the copolymers present a better thermal stability when compared to unmodified PVA.

The copolymers were synthesized to be subsequently used as matrices for achievement of nanocomposites with hydroxyapatite or argyles. The raw materials utilized in the synthesis are biodegradable, biocompatible products and are commercially available. As a result of researches done concerning biodegradation as well as on the basis of analysis of catalase activity (considered as a biochemical marker to establish the biodegradation degree), from the fungus micelle *Chaetomium globosum* it was found that the copolymer films present a raised degree of biodegradation. In reality these could be considered for the achievement of composite materials with important applications. As the copolymers melt on temperature interval between 140-200 ^{0}C, they can be processed by moulding, comparative to PVA that decomposes at approximate 230 ^{0}C.

Zeta potential measurements can be a useful tool for characterization of colloidal dispersions, especially when used in conjunction with other techniques, such as particle size distribution. They can give information on the surface properties of carriers, size distribution of particles, stability of colloidal solutions, the association and organization manner of different molecules in a system, and their release in different media.

Experimental part

Materials

The raw materials used in our experiments were available as commercial products. Poly(vinyl alcohol) Mowiol® 4-88 from Hoechst (Germany) had a degree of hydrolysis 88 mol.%, degree of polymerization 630, average molecular weight Mw=31000, ester number 130-150 mg KOH/g. L(+)–lactic acid [(S)-2-hydroxypropionic acid] 90 wt.% aqueous solution, density 1.20 g/mL from (Fluka, Switzerland), and L-aspartic acid [(S)-2-aminobutanedioic acid] Merck (Germany) were used as received. Manganese acetate, dioxane, N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO) from Fluka (Switzerland), were analytical grade reagents. Acetone, methanol, ethanol, ethylene glycol from Chimopar S.A. (Romania), and ammonium hydroxide aqueous solution 25 wt.% from Chemical Company Iassy (Romania), were used without further purification.

Synthesis

The syntheses of PVA-LA-Asp copolymers were carried out by solution polycondensation procedure, in a three necked round bottom flask equipped with heating system, and distillation-collecting device (Dean-Stark trap) of the secondary reaction products. The composition of the initial reaction mixture and the reaction conditions are given in Table 7. The reactions took place under blanket of nitrogen and different molar ratios PVA/LA/Asp: 1/1/0.075 (sample A); 2/1/0.075 (B); 1/2/0.075 (C); 1/1/0.05 (D); 2/1/0.05 (E); 1/2/0.05 (F), were used. Molar ratios of lactic acid and aspartic acid were related to the monomeric unit of PVA ($–CH_2–CH–OH–$). Manganese acetate [$Mn(CH_3COO)_2·4H_2O$] as catalyst (0.8 wt.%, reported to the reaction components), and dioxane/water (3/1 v/v) as azeotrope solvent mixture, were used. Manganese acetate is a new catalyst rarely utilized in the polycondensation reactions of lactic or aspartic acid, and its utilization is justified as it presents very reduced toxicity [35]. It is sometimes used as a food additive and as packaging material for the food industry. PVA was dissolved in water at 60 ^{0}C temperature, and solutions of 17 wt.% were obtained. Then, the adequate quantity of L(+)-lactic acid (90 wt.% aqueous solution), aspartic acid dissolved in NH_4OH (25 wt.% aqueous solution), and finally the adequate quantity of catalyst dissolved in distilled water, were introduced under continuous stirring. The reaction took place in two stages: in the first stage the temperature was maintained at 85-90 ^{0}C for 3 hours with reflux, then an equipment of distillation-collection was assembled and the temperature was raised for 5 hours at 110-120 ^{0}C and developed with secondary products removal (water, dioxane, unreacted lactide). After cooling at room temperature, the reaction product was precipitated with acetone in order to remove PLA homopolymer and a white colour precipitate was obtained. After decantation and purification several times with acetone, the product was dried under vacuum at 80 ^{0}C for 24 hours, afterwards was ground. The yield of the synthesized copolymers varied between 25.5 and 77%, the highest yields values were obtained at molar ratio PVA / LA (2/1).

Tab. 7. The composition of the initial reaction mixture and the reaction conditions.

Copolymer	PVA mol	LA mol	Asp mol	Yield %	Time/temperature reaction
A	1	1	0.075	46	3h/85-90 ^{0}C 5h/115-120 ^{0}C
B	2	1	0.075	77	
C	1	2	0.075	39	
D	1	1	0.05	48	
E	2	1	0.05	61.5	
F	1	2	0.05	25.5	

Conditions: catalyst [$Mn(CH_3COO)_2\cdot 4H_2O$] (0.8 wt.%, reported to the reaction components); dioxane/water (3/1 v/v) as azeotrope solvent mixture.

Measurements

FTIR spectra were recorded using a **Bruker** Vertex 70 spectrophotometer (Germany). The samples were homogenized and pressed on KBr pellets.

^{1}H-NMR spectra were recorded by Bruker Avance DRX 400 NMR spectrophotometer (Germany) equipped with 9.4 Tesla (400 MHz) ultrashield magnet. The samples were dissolved in D_2O at 40 ^{0}C.

The average particle size and zeta potential were measured using a Zetasizer Nano-ZS, ZEN-3500 model, (Malvern Instruments, Malvern UK). Particle size and polydispersity were determined using noninvasive back scatter (NIBS) technology, which allows sample measurement in the range of 0.6 nm-6 μm. For the measurement, the copolymers were dissolved in bidistilled water at a concentration of 0.01g/L. The measurement was carried out using a 50 mW He-Ne laser green light (532 nm) as light source at a fixed angle of 173^0. The following parameters were used for experiments: water as dispersant medium, medium refractive index RI=1.330, medium viscosity 0.887 cP, a dielectric constant of 79, temperature 25 ^{0}C. All measurements were carried out in triplicate directly after sample preparation, and the results were expressed as mean size. After the size measurement, zeta potential was measured with the M3-PALS technique, a combination of Laser Doppler Velocimetry (LDV) and Phase Analysis Light Scattering (PALS). A Smoluchowsky constant f(ka) of 1.5 was used to calculate zeta potential from the electrophoretic mobility. Each zeta potential measurement was performed automatically at 25 ^{0}C. All measurements were carried out in triplicate directly after sample preparation, and results were expressed as mean zeta potential.

Thermogravimetric analyses (TG nd DTG) were carried out using a derivatograph Q-1500 D MOM Budapest (Hungary) at the following operational conditions: the weight of the sample 50 mg, the heating rate 10 ^{0}C/min, the maximum heating limit 700 ^{0}C, reference material α-Al_2O_3.

DSC thermal analyses were carried out by means of a differential scanning calorimeter Perkin Elmer Pyris Diamond with a heating rate of 10 ^{0}C/min in nitrogen atmosphere. The heating run was carried out with a 10-12 mg sample in the temperature range between -20 ^{0}C and 235 ^{0}C. Pure indium was used as a standard for calorimetric calibration.

Elemental analyses (C, H, N) were recorded on Perkin Elmer Analysis 2400 apparatus.

Wide angle X-ray diffraction (WAXD) was recorded by Bruker AXS D8 Advance diffractometer with scintillation detector in Bragg – Bretagne geometry, copper anode, X-ray tube type Siemens KFL-Cu 2K, at U<60 kV and 50 mA emission current.

Biodegradability tests were achieved in laboratory conditions, on agar-agar medium, inoculated with fungus. The results were average value of three parallel experiments.To achieve the biodegradation study of the PVA-LA-Asp copolymers, films with thickness of 100-200 μm were used, obtained by melting and pressing procedure (160-170 ^{0}C) between two polytetrafluorethylene layers. Films obtained from the PVA-LA-Asp copolymers were exposed on agar-agar medium, inoculated with a fungus of *Chaetomium globosum* class, MO 5 stock. The fungus was cultivated on Heynes medium (yeast extract 4 g, malt extract 10 g, dextrose 4 g, agar-agar 15 g, distilled water 1000 ml) and after 7 days of seeding on these cultures the films were applied. Temperature was maintained at 28 ^{0}C, and the biodegradation monitoring was carried out after 4 and 14 days from the film application.

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