

Chapter III

Results and Discussion

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3.1. Graft Copolymerization of N-vinylpyrrolidone onto poly (3-hydroxy butyrate-co-3-hydroxyvalerate)

3.1.1. Evidence of grafting of PVP onto PHBV.

The infrared spectra of PHBV, PVP and representative grafted PHBV copolymers are shown in Fig.7. For PHBV, the carbonyl vibration consists of a peak centered at 1723 cm^{-1} and a shoulder at 1740 cm^{-1} , which are assigned to the vibrations of crystalline and amorphous carbonyl ester groups of the repeat units, respectively¹²⁶.

For all the graft copolymers a new absorption band was found in the region $1656\text{--}1660\text{ cm}^{-1}$ which is assigned to the amide C=O however, it occurs at lower frequencies than “normal” carbonyl absorption due to the resonance effect (amide I). I also find C–N stretching band in the $1440\text{--}1200\text{ cm}^{-1}$ region. These bands are however to a large extent overlaid by the C–H and CH₂– and CH₃– bands in this region stemming from the main chains of the PHBV and PVP graft chain.

Fig. 8 shows ¹H-NMR spectra of the PHBV and the grafted PHBV-22g-PVP as representative examples with their peak assignments. Through comparison of the spectrum of pure PHBV with that of the graft copolymer, the most striking characteristics peaks in the spectrum of the graft sample, denoted by [g], [k], [h] at 2.03, 2.33 and 3.18 ppm, are assigned to the methylene group of the pyrrolidone ring, and the peak at around 3.74 ppm denoted as [f] corresponding to the methine proton of the repeating PVP unit.

Peaks corresponding to PHBV were observed and were consistent with the assigned structure.

3.1.2. Influence of reaction conditions on the extent of grafting

The grafting of N-vinylpyrrolidone onto PHBV in homogeneous medium was optimized by changing the initial concentration of monomer, initiator, and reaction time.

3.1.2.1. Effect of monomer concentration

The effect of monomer concentration on the grafting reaction was studied at various concentrations of VP while other factors were kept constant Fig. 9. As can be clearly seen in Fig. 9, it can be seen that the extent of grafting and the grafting efficiency were increased with increasing VP monomer concentration to a maximum of 42 % at around 2 M and thereafter they were rapidly decreased., showing that higher VP concentrations do not enhance further grafting which may be explained by increasing the homopolymerization tendency. The relatively low grafting extent could be due to the high crystallinity of the base polymer

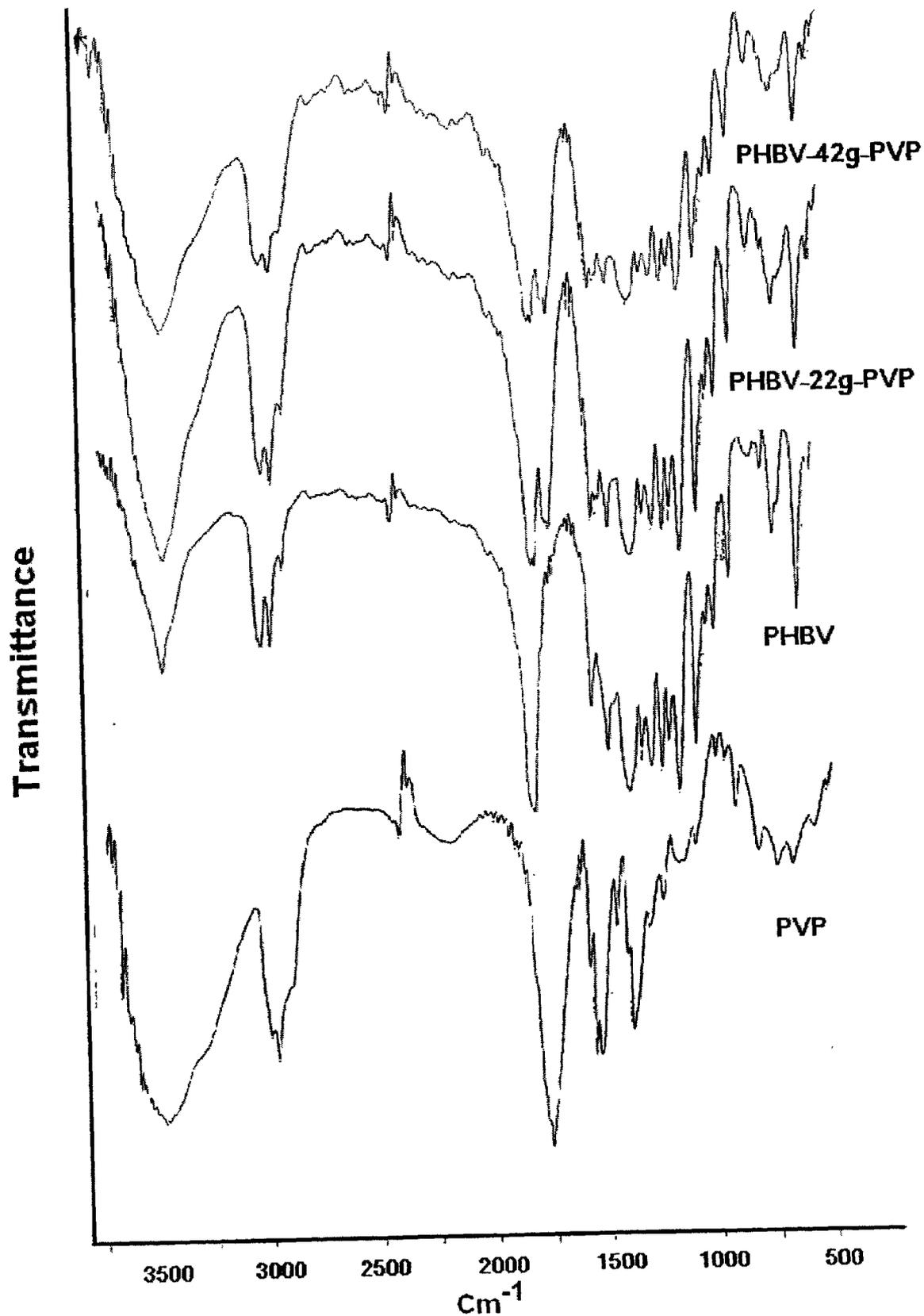


Figure 7: FTIR spectra of PHBV, PVP and graft copolymers (22, 42%G)

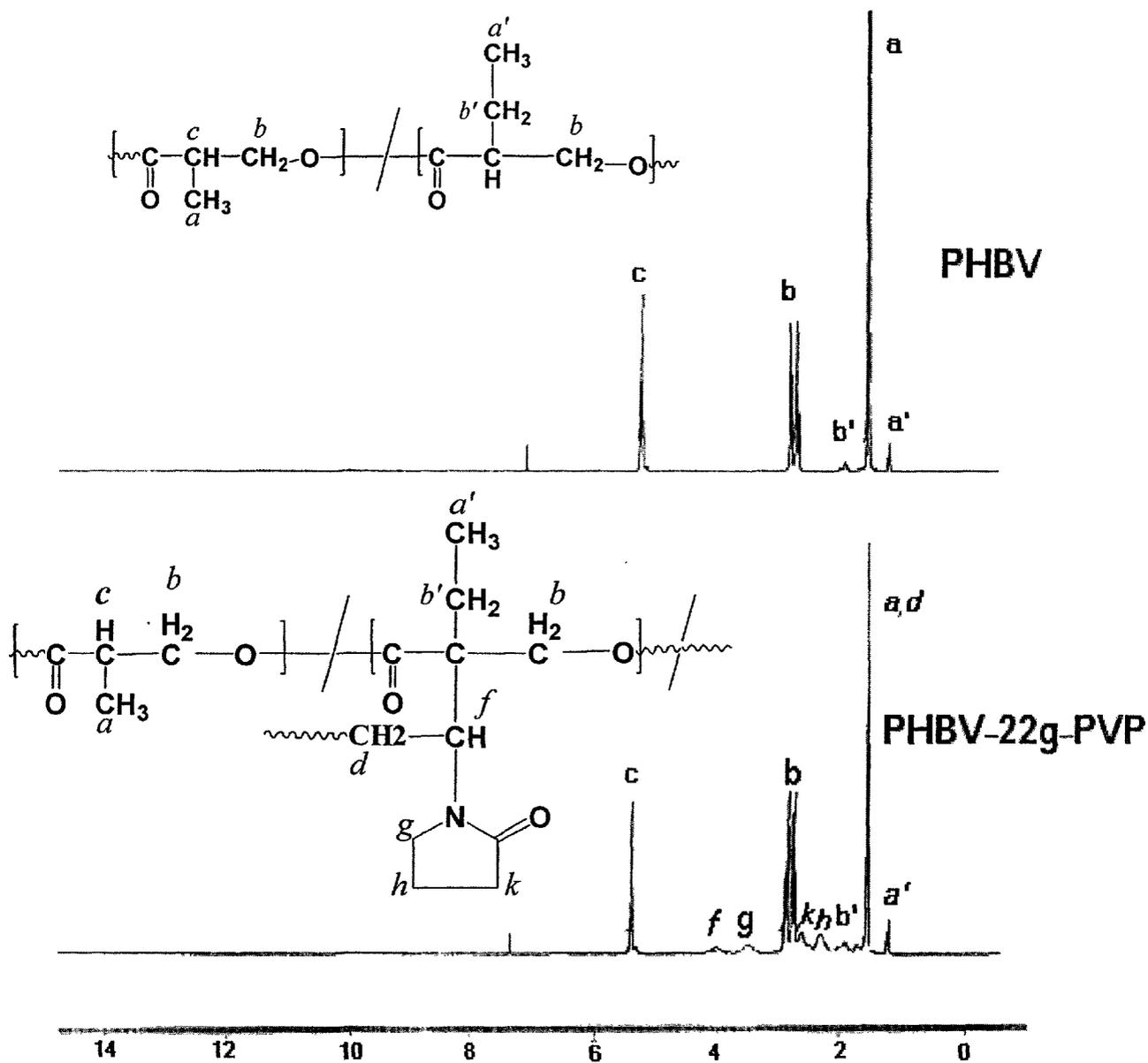


Figure 8: ¹H-NMR spectra of PHBV and PHBV-22g-PVP graft sample.

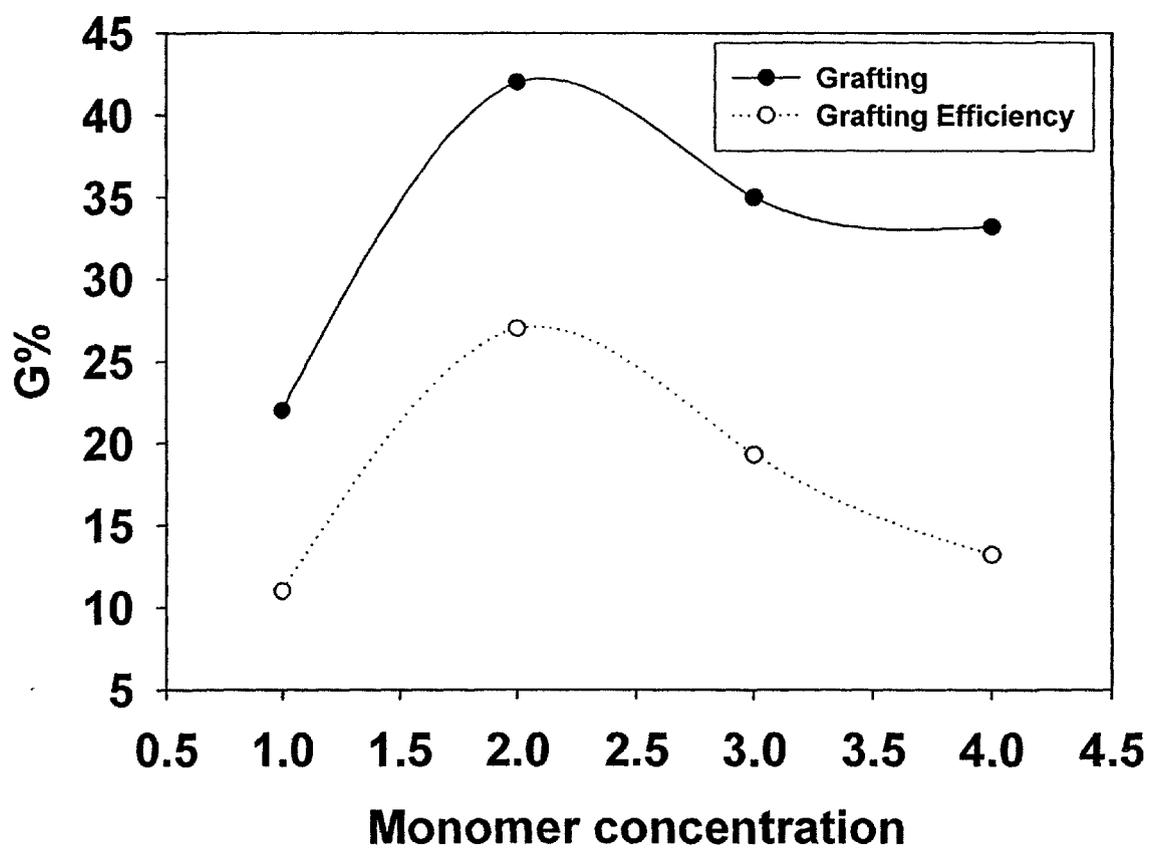


Figure 9: Effect of monomer concentration on the grafting reaction, $[I] = 4.4 \times 10^{-3} \text{ M}$ and $T = 40^\circ\text{C}$

3.1.2.2. Effect of initiator concentration

Fig. 10 shows the dependence of the extent of grafting on the initiator concentration. The results indicate that the extent of grafting and the grafting efficiency were increased with increasing initiator concentration until it reaches a maximum value of around 42% grafting at 4.4×10^{-3} M of AIBN. A further increase in initiator concentration is accompanied by a considerable decrease in the grafting percent. This could be due to the increased probability of the termination reaction, through the combination of the growing active chains.

3.1.2.3. Effect of reaction time

The influence of reaction time on the extent of grafting is shown in Fig. 11. Increasing the reaction time leads to an increase in the extent of grafting and the grafting efficiency. At longer reaction times, the percentage of both grafting and grafting efficiency begins to decrease. This is presumably due to the depletion of the monomer, initiator and available grafting sites as the reaction proceeds, or could be due to degradation of the host polymer with time at the refluxing temperature.

3.1.3. Viscosity Measurements

Fig. 12 shows the intrinsic viscosity of PHBV and its graft copolymers. A gradual decrease in the value of $[\eta]$ is observed with increasing the extent of grafting. This decrease may be due to main chain scission during the grafting process; since the polymerization is carried out in at reflux temperature in methylene chloride beside the long reaction duration rang of [12 – 48h]

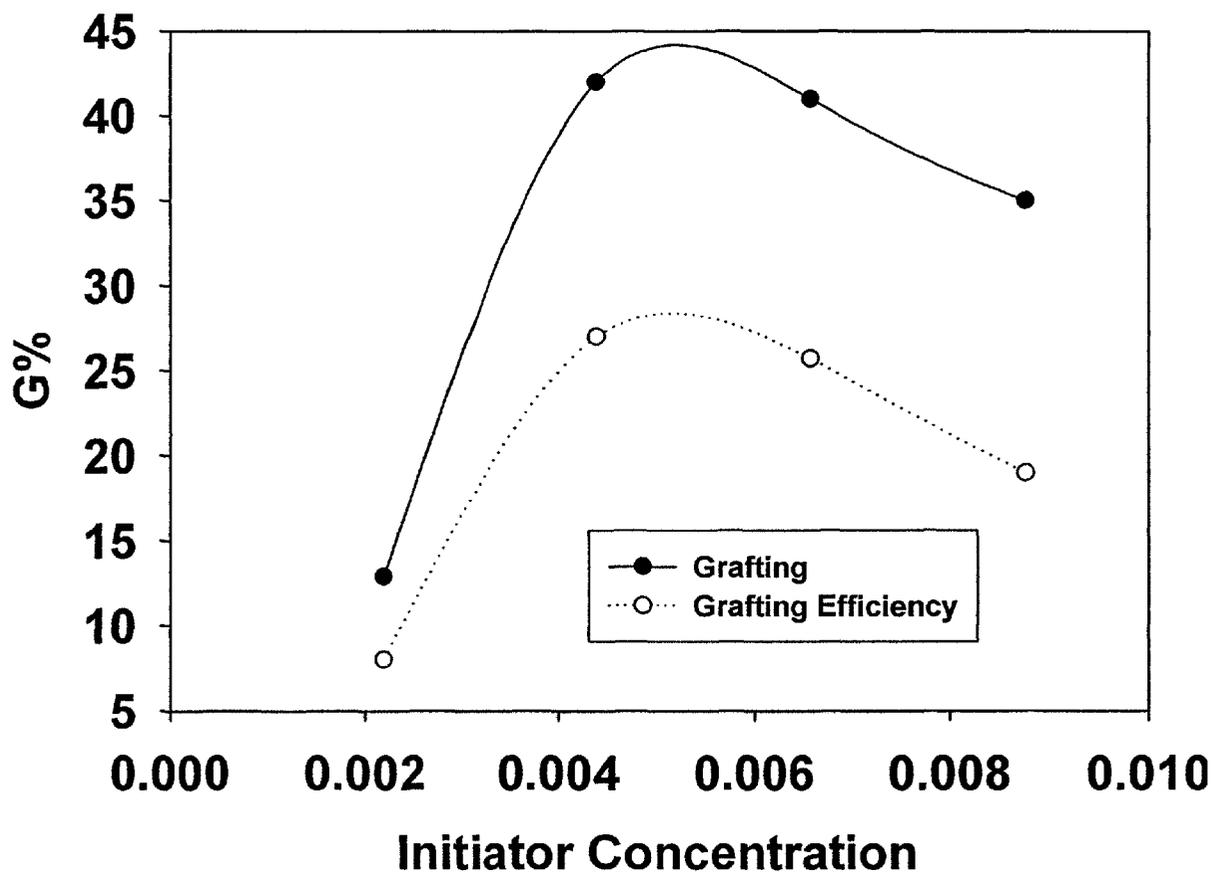


Figure 10: Effect of initiator concentration on the grafting reaction, $[M] = 2 \text{ M}$, $T = 40^\circ\text{C}$

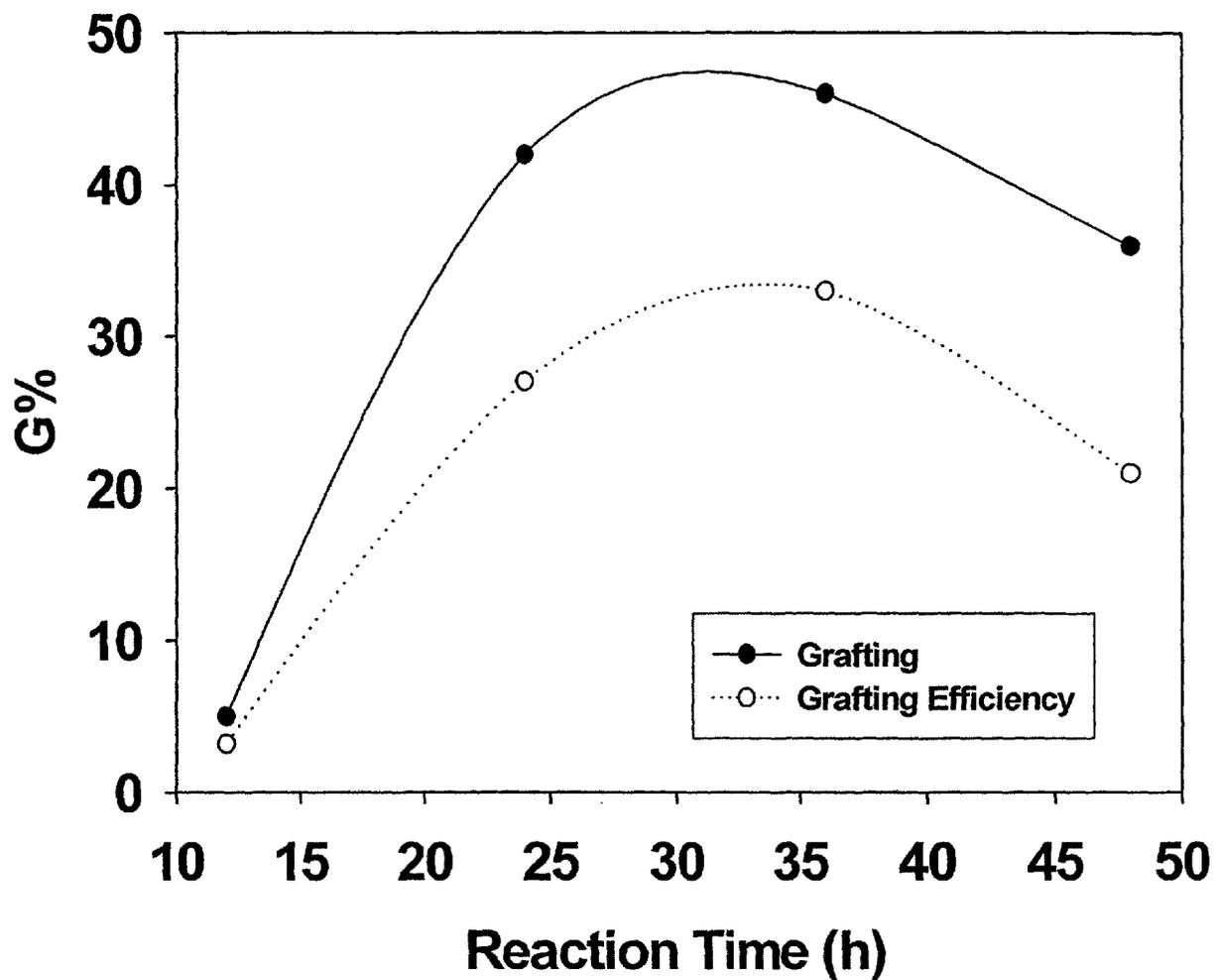


Figure 11: Effect of time on the grafting reaction $[M] = 2 \text{ M}$, $[I] = 4.4 \times 10^{-3} \text{ M}$, $T = 40^\circ\text{C}$

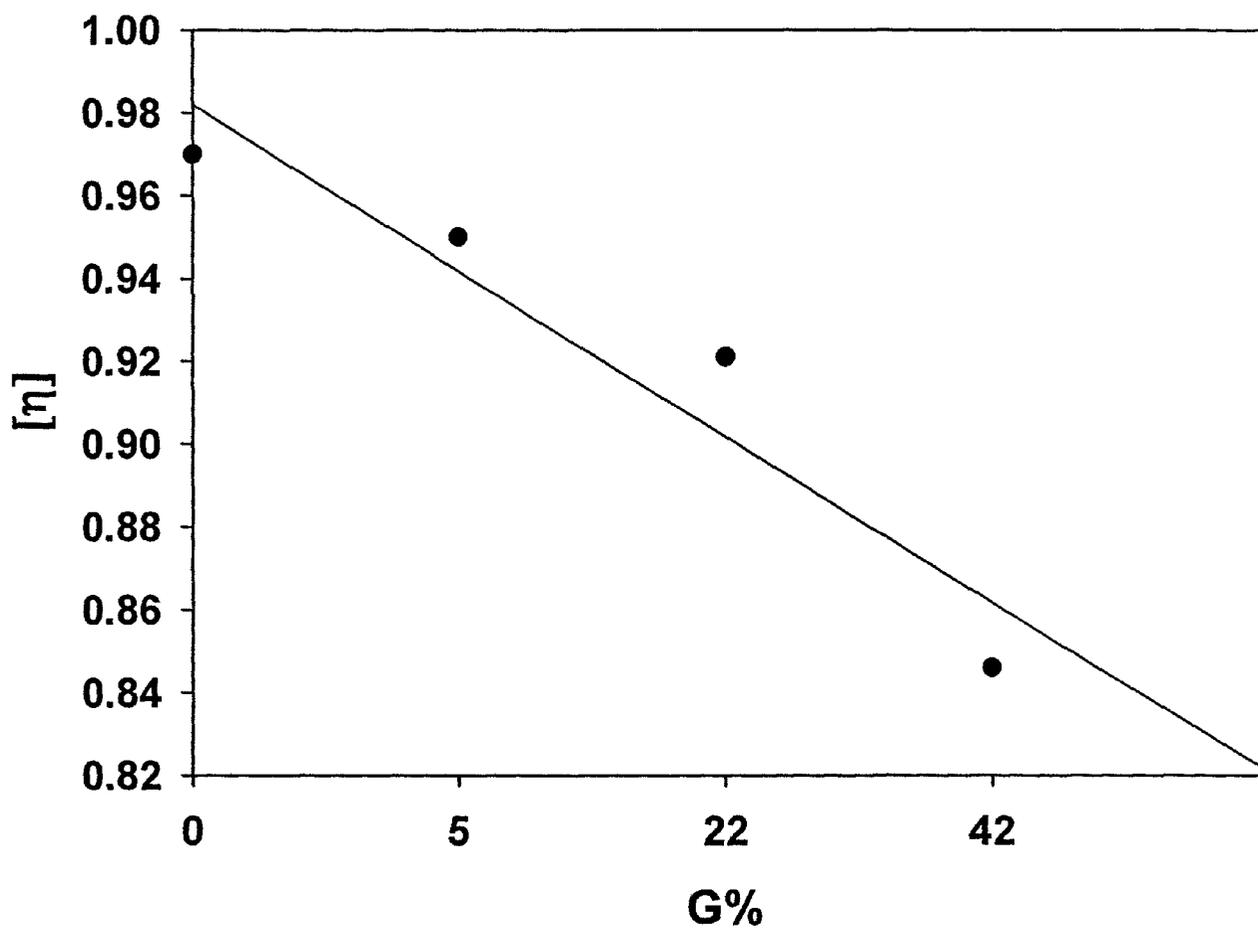


Figure 12: The effect of the grafting of VP onto PHBV on the intrinsic viscosity

3.1.4. Thermal analysis

Fig.13 (a) shows the DSC curves of PHBV and some of the grafted copolymers recorded after quenching. The main thermal transitions of all the investigated samples are summarized in Table 1. It was found that cold crystallization temperature (T_{cc}) values of the PHBV graft samples are shifted systematically to higher temperature with increasing the extent of grafting. This indicates that introduction of PVP chains can retard the crystallization of PHBV because the regularity of PHBV chains is, to a certain degree, disturbed after grafting with PVP. From the data in Table 1, it is found that the T_g values of the grafted samples are almost constant, irrespective of the extent of grafting. The original PHBV shows double melting peak. The peak appearing at higher temperature is assigned to the melting of the reorganized crystal formed during DSC heating process, while the lower temperature one, which appears as a shoulder, corresponds to the fusion of the crystallites formed on cooling from the melt¹²⁷. From Figure 13(a) and Table 1 one can notice that the lower peak is affected by grafting while that the peak at higher temperature is almost constant. This could be a consequence of grafting since the side graft chains could interfere with the crystallization process. These observations are consistent with the data in literature⁸⁷, the melting enthalpy (ΔH_m) slightly decreased (Table 1) with the increase in the graft degree, which implies that after grafting with PVP, the crystallinity of PHBV declines.

The DSC thermograms of the copolymers recorded after slow cooling from the melt and annealing for one week at room temperature Figure. 13(b) showed neither glass transition nor exothermic crystallization peaks, indicating that slow cooling and annealing would allow complete crystallization. The figure also indicates that the higher

Table 1 Thermal characteristics of PHBV and its graft copolymers with PVP

Sample code	T_m (°C)	T_g (°C)	T_{cc} (°C)	ΔH_m (J/g)	Xc%
PHBV	158.4, 170.7	7.7	64.6	67.0	45.9
PHBV-5g-PVP	156.7, 171.2	7.9	70.0	63.1	45.4
PHBV-17g-PVP	155.5, 168.2	7.4	71.9	52.3	43.0
PHBV-22g-PVP	150.8, 170.0	8.5	72.31	46.0	40.3
PHBV-35g-PVP	141.9, 169.9	8.3	74.39	36.8	38.7
PHBV-42g-PVP	140.0, 168.3	8.6	77.46	30.1	35.4

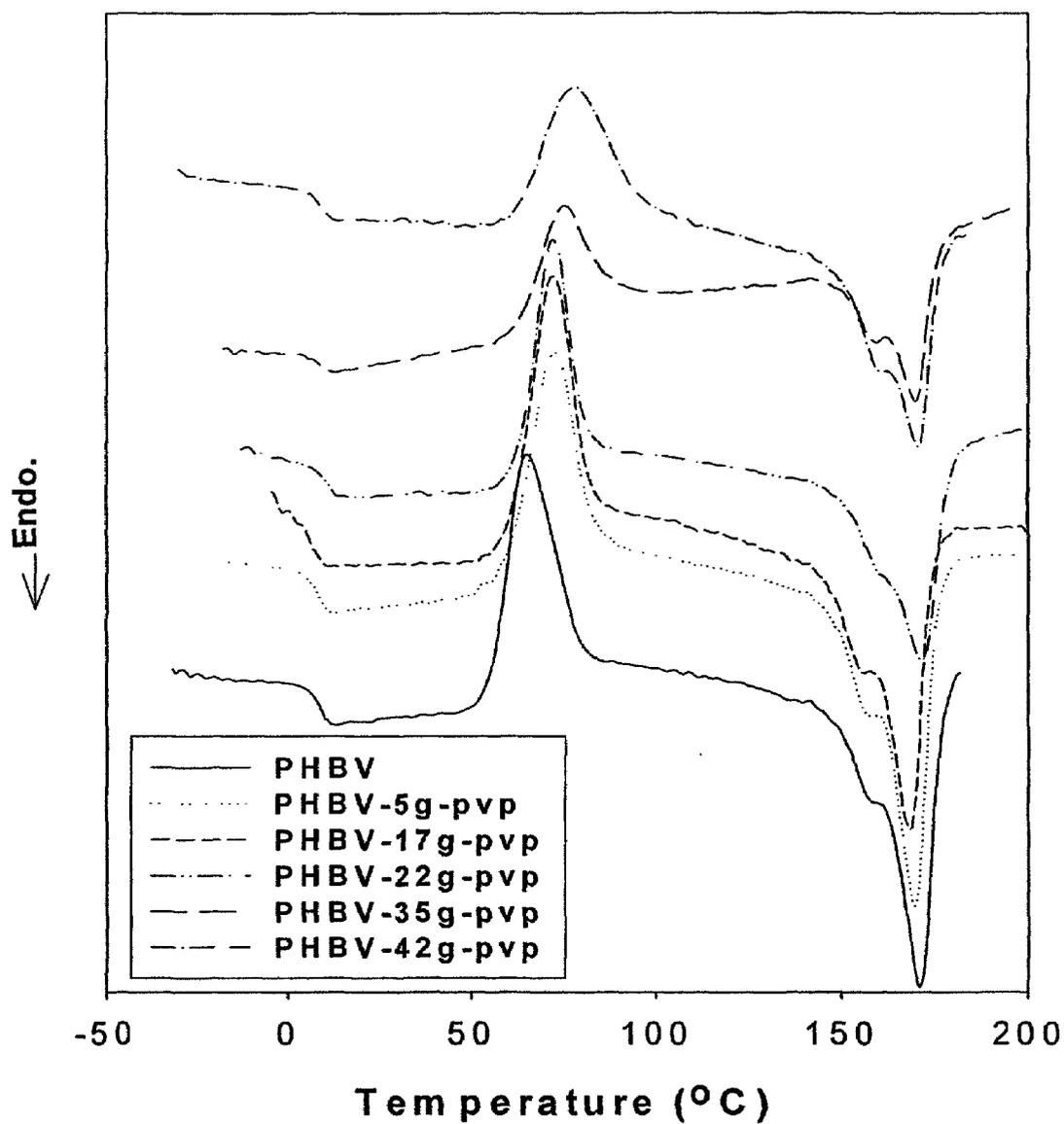


Figure 13(a): DSC curves of PHBV and some grafted copolymers recorded after quenching (run II)

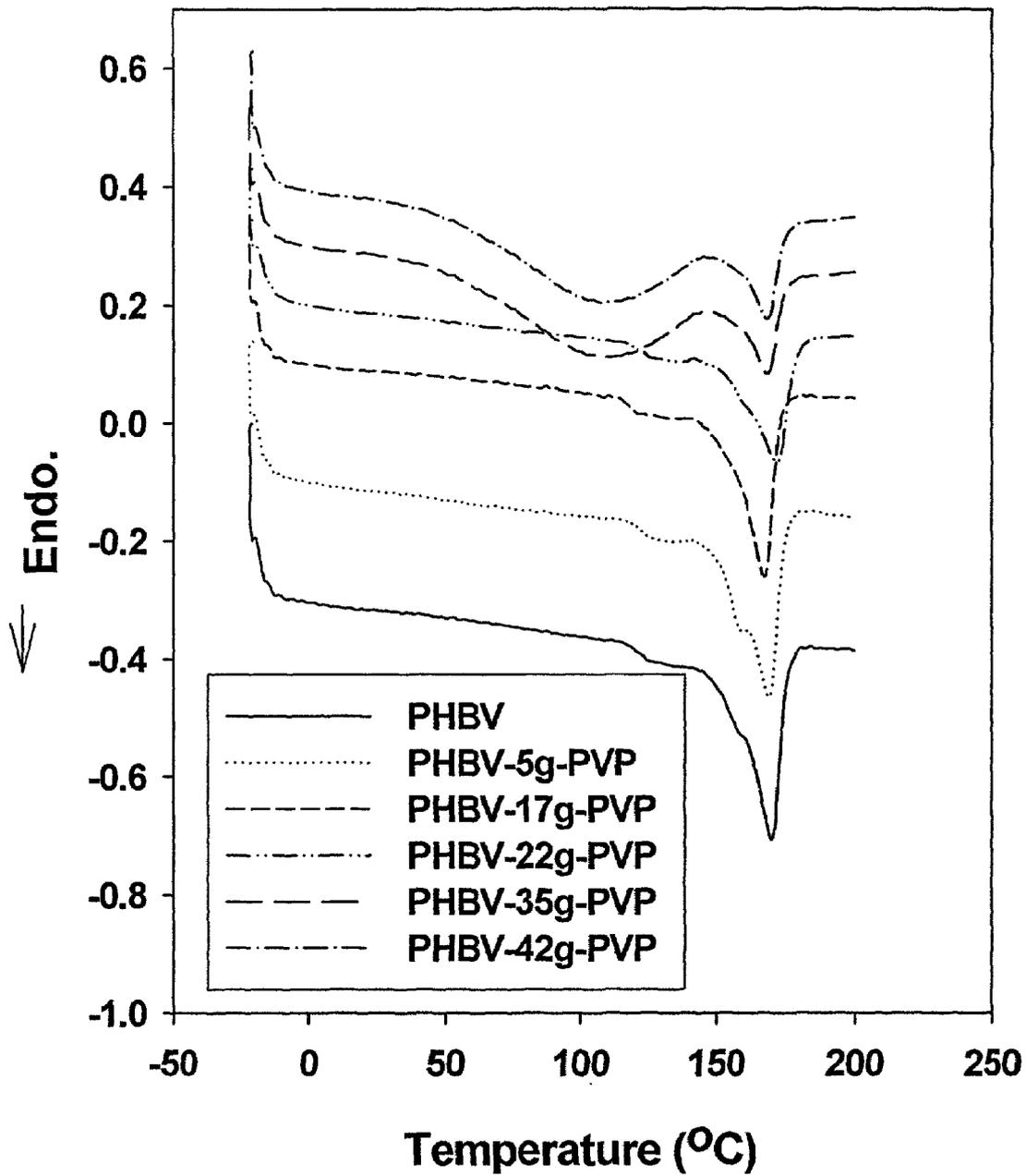


Figure 13(b): DSC curves of PHBV and some grafted copolymers recorded after annealed for one week at room temperature (run III)

grafted samples absorb water which is reflected by the broad endothermic peaks around 110°C.

The normalized heat of fusion (melting enthalpy), i.e., the value calculated for 1 g of the PHBV, instead of 1 g of copolymer, is used to estimate the relative crystallinity percent (X_c %) of PHBV base polymer.

$$X_c \% = \frac{\Delta H_m}{(\Delta H_m^\circ \cdot W)} \cdot 100 \quad (5)$$

where ΔH_m is the heat of fusion corresponding to PHBV crystalline phase, ΔH_m° is the heat of fusion of 100% crystalline PHBV; the literature value being 146 J/g¹²⁸. W is the weight fraction of PHBV in the graft copolymer.

Computed results are included in Table 1, and revealed that the degree of crystallinity was slightly decreased by PVP graft chains. This implies that the grafting of VP monomer occurred mainly in the amorphous region of main chain polymer.

Fig. 14 illustrates the TGA and DTGA of the PHBV-22g-PVP and PHB-42g-PVP copolymers, the thermogram of pure PHBV is included in the figure for the sake of comparison. In contrast to PHBV copolymer, whose thermal degradation takes place as a single step in the temperature range 260 to 350°C¹²⁹, the decomposition of the grafted PHBV polymers is more complex.

The decomposition of the graft copolymers takes place by three steps in addition to water loss absorbed on the PVP graft chains around 110°C. The main decomposition temperature takes place at about 290°C followed by two smaller peaks at around 400-450 and 490-550°C depending on the extent of grafting. The first peak is probably due to

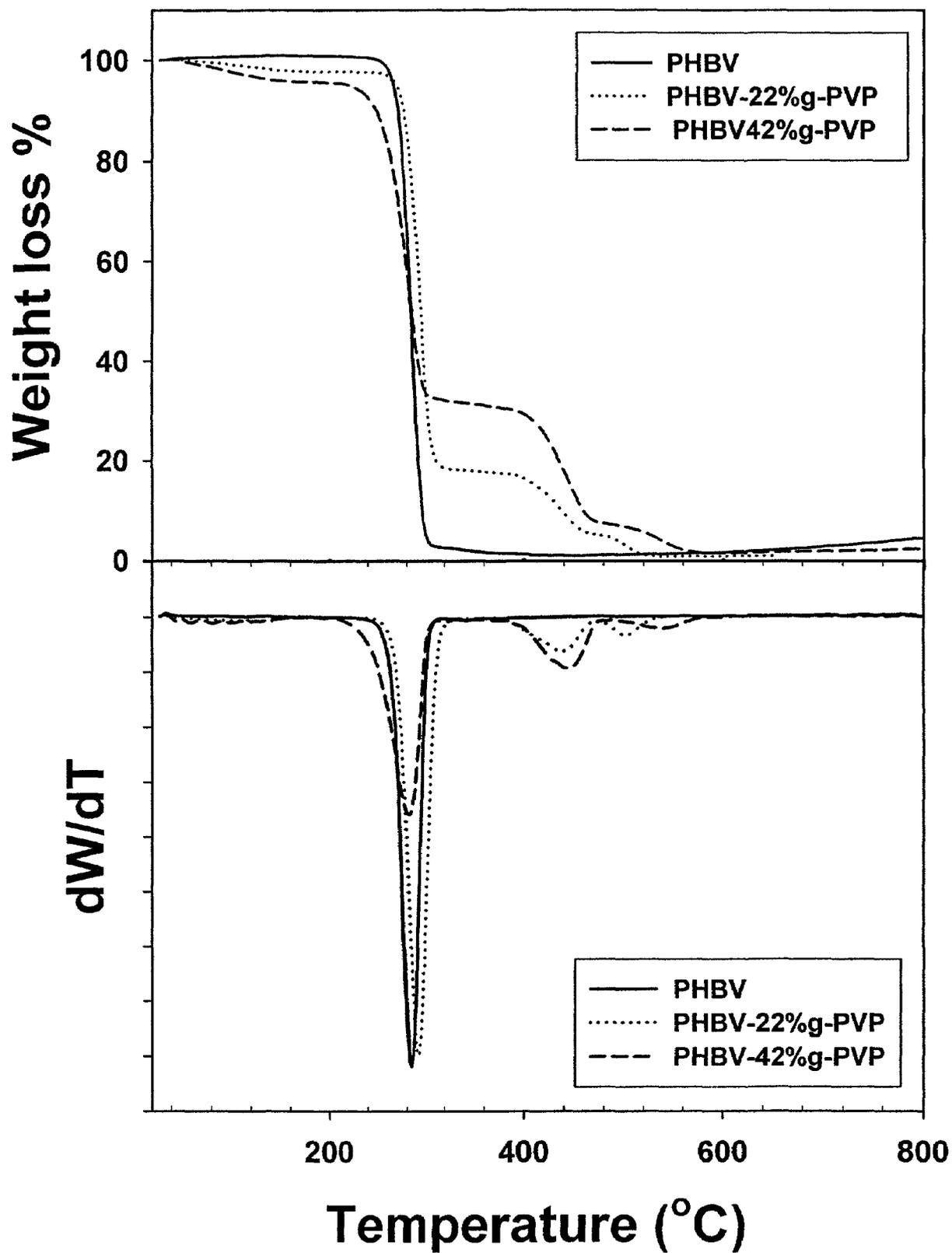


Figure 14: TGA and DTGA of PHBV and some grafted copolymers

PHBV main chain decomposition, while the others are due to PVP graft chains. It can be seen that grafting of the host polymer did not affect much its thermal stability.

3.1.5. Wide angle XRD analysis

Fig. 15 shows the X-ray diffraction patterns of PHBV and PHBV-g-PVP samples. The diffraction patterns of PHBV consisted of two major crystalline peaks at $2\theta = 13.5^\circ$ of the (020) plane and 16.9° of the (110)^{25, 130}. After grafting, the positions of these two peaks and the corresponding relative intensity ratios did not change significantly even when the extent of grafting reached 45%, indicating that the crystalline structure of PHBV is not changed with grafting. This confirms that PVP chain branches formed only in the non-crystalline regions of PHBV.

3.1.6. Swelling behavior

Polyesters are generally hydrophobic materials. However, grafting of PHBV with PVP enhances dramatically the swelling ability of the final graft in distilled water as shown in Fig 16 (G% = 5, 22, 35, 46). Fig. 16 shows that equilibrium swelling was achieved after about 20 min. The Figure shows the dependence of the swelling degree at equilibrium as a function of the extent of grafting. It can be seen that pure PHBV almost has no affinity for water. Sample with higher grafting shows enhanced swelling compared with the sample of low grafting.

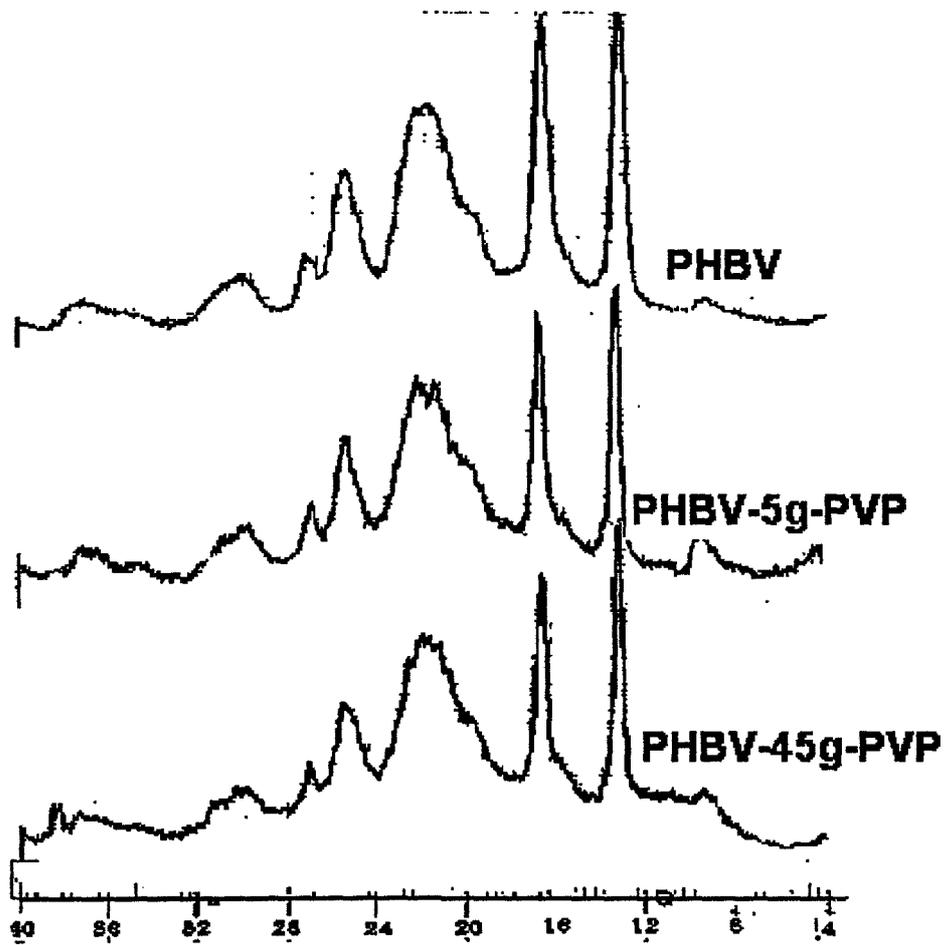


Figure 15: Wide-angle X-ray curves for of PHBV and some grafted copolymers

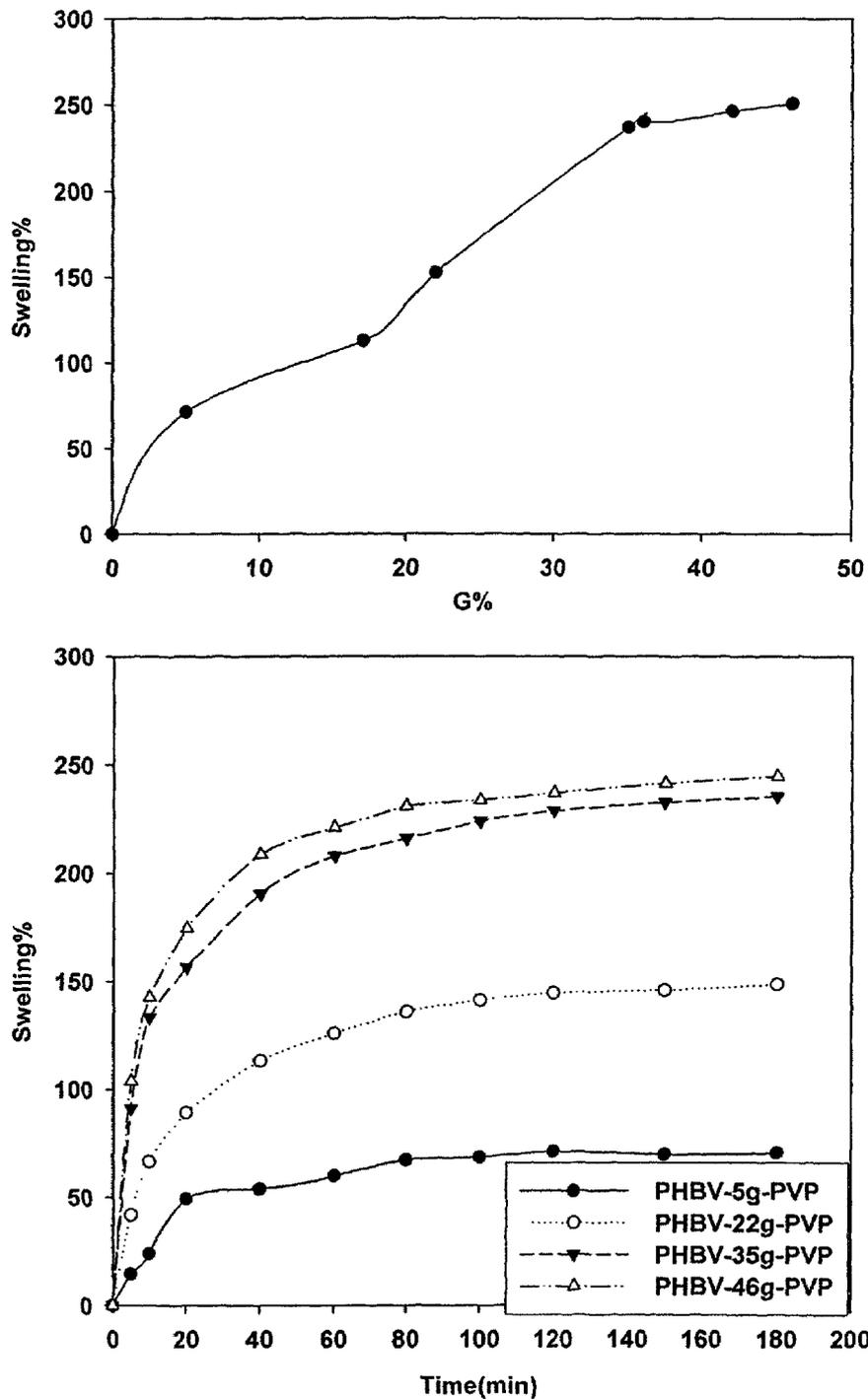


Figure 16: Swelling of PHBV- g-PVP copolymer at different grafting percent ($G\% = 5, 22, 35,$ and 46) as a function of time in distilled water ($\text{pH}, 6.5$), Temperature = 30°C and Effect of percent of grafting on the equilibrium swelling extent.

3.1.7. Antimicrobial activity

Polyvinyl pyrrolidone has been used extensively in the biomedical field from this point of view a question arises whether it has any antibacterial and anti microbial properties? This promoted the necessity to explore whether grafting of PHBV, which is known to have no antibacterial and antifungal characteristics, with PVP would impart any antimicrobial behavior for the new graft copolymers. Therefore Gram positive, Gram negative, and some yeast and filamentous fungi were used to answer this question. The tested fungal and bacterial species were selected for their significant role as causative agents of animal and human diseases, and some dermatophytes organisms.

The screening of the investigated compounds for their antimicrobial activities was carried out by two methods, namely the agar well and the minimal inhibitory concentration (MIC_S) methods. The degree of antimicrobial activity of the antagonistic bioactive materials was evaluated according to Abdel-Fattah et al.¹³¹, who classified the antagonistic activity of microorganisms, depending on the mean diameters of inhibition zones in mm to the following groups: very weak activity (<16 mm), weak activity (16-19 mm), moderate activity (20-29 mm) and good activity (30 mm or more)

The results, obtained using the above two methods, among the selected bacteria and fungi are presented in Table 2-5. From Table 2 and 3 one can see that the biological activity of the graft copolymers increased with increasing the extent of grafting, as reflected by increasing the inhibition zone, for the tested fungi and bacteria. This may be due to the inhibition of the glucose uptake, inhibition of RNA and induced cell death for the microorganisms.^{132, 133} The results also show that the degree of the biocidal activity of the investigated compounds depends on the tested microorganisms and degree of

grafting. The sensitivity of the fungi toward the sample of 46% PVP graft (PHBV-g-PVP) increases in the following order:

Fusarium oxysporum ~ *Aspergillus niger* > *Aspergillus flavus* ~ *Saccharomyces cerevisiae* ~ *Candida albicans* > *Candida tropicalis*. > *Candida nonalbicans* > *Candida parapsilosis*.

The results also revealed that the growth inhibition of the bacteria for the investigated compounds follows the order:

Bacillus subtilis ~ *Micrococcus luteus* > *Salmonella typhimurium* ~ *Staphylococcus aureus* ~ *Escherichia coli* ~ *Pseudomonas aeruginosae* ~ *Shigella spp* ~ *Klebsiella pneumonia*

The results summarized in Table 4 and 5, obtained by using the minimal inhibitory concentration method (MIC_S) method for the tested organisms, have the same trend.

Table 2: Antifungal activity data (inhibition zone, mm) of the grafted copolymer on the tested fungi

Grafted compound		Tested organisms used							
		<u>F.</u> <i>oxysporum</i>	<u>A.</u> <i>niger</i>	<u>A.</u> <i>Flavus</i>	<u>S.</u> <i>cerevisiae</i>	<u>C.</u> <i>Albicans</i>	<u>C.</u> <i>tropicalis</i>	<u>C.</u> <i>Non-albicans</i>	<u>C.</u> <i>parapsilosis</i>
PHBV		8	7	6	6	6	7	6	7
PHBV-5g-PVP		17	16	15	13	12	15	11	14
PHBV-22g-PVP		20	19	19	17	16	18	15	16
PHBV-42g-PVP		24	21	20	18	20	19	16	19
PHBV-46g-PVP		38	34	35	33	36	35	26	32
LSD	5%	0.71	0.62	0.53	0.42	0.41	0.52	0.61	0.56
	1%	1.63	1.48	1.47	1.36	1.43	1.53	1.44	1.60

Table 3: Antibacterial activity data (inhibition zone, mm) of the grafted copolymer on the tested bacteria

Grafted compound		Tested organisms used							
		<u><i>B. subtilis</i></u> (Gm +ve)	<u><i>S. typhimurium</i></u> (Gm -ve)	<u><i>S. aureus</i></u> (Gm +ve)	<u><i>M. luteus</i></u> (Gm +ve)	<u><i>E. coli</i></u> (Gm -ve)	<u><i>P. aeruginosa</i></u> (Gm -ve)	<u><i>Shigella spp.</i></u> (Gm -ve)	<u><i>K. pneumoniae</i></u> (Gm -ve)
PHBV		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
PHBV-5g-PVP		17	13	18	21	39	18	14	22
PHBV-22g-PVP		22	16	27	28	34	22	20	29
PHBV-42g-PVP		25	20	32	36	42	33	23	31
PHBV-46g-PVP		48	48	38	37	45	36	36	34
LSD	5%	0.83	0.66	0.56	0.47	0.56	0.62	0.67	0.60
	1%	1.77	1.57	1.59	1.41	1.59	1.81	1.84	1.74

Table 4: The minimum inhibitory concentration (MIC_S, µg/ ml) of the grafted copolymers on the tested fungi

Grafted compound		Tested organisms used							
		<u>F.</u> <i>oxysporum</i>	<u>A.</u> <i>niger</i>	<u>A.</u> <i>Flavus</i>	<u>S.</u> <i>cerevisiae</i>	<u>C.</u> <i>albicans</i>	<u>C.</u> <i>tropicalis</i>	<u>C.</u> <i>Non-albicans</i>	<u>C.</u> <i>parapsilosis</i>
PHBV		750	750	750	1000	1250	1250	1250	1250
PHBV-5g-PVP		450	475	475	450	525	550	600	625
PHBV-22g-PVP		375	375	400	400	425	425	450	450
PHBV-42g-PVP		250	275	375	375	375	375	400	400
PHBV-46g-PVP		125	125	150	150	150	175	200	250
LSD	5%	3.31	3.86	3.91	4.13	4.84	5.21	5.62	5.81
	1%	7.41	8.40	9.41	10.44	11.22	12.10	13.71	14.40

Table 5: The minimum inhibitory concentration (MIC_s, µg/ ml) of the grafted copolymers on the tested bacteria

Grafted compound		Tested organisms used							
		<u><i>B. subtilis</i></u> (Gm +ve)	<u><i>S. typhimurium</i></u> (Gm -ve)	<u><i>S. aureus</i></u> (Gm +ve)	<u><i>M. luteus</i></u> (Gm +ve)	<u><i>E. coli</i></u> (Gm -ve)	<u><i>P. aeruginosa</i></u> (Gm -ve)	<u><i>Shigella spp.</i></u> (Gm -ve)	<u><i>K. pneumoniae</i></u> (Gm -ve)
PHBV		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
PHBV-5g-PVP		175	200	225	225	275	275	350	275
PHBV-22g-PVP		150	175	175	175	200	200	250	200
PHBV-42g-PVP		50	75	75	75	100	100	125	100
PHBV-46g-PVP		25	50	50	25	50	50	50	50
LSD	5%	4.40	4.91	5.81	6.70	6.81	7.82	8.21	7.91
	1%	9.81	10.42	12.70	14.31	17.30	18.41	17.40	18.97

(-) Non detectable and behaves as preserved food material for bacteria

3.1.8. Biodegradability of PHBV and PHBV-g-PVP

Environmentally, the most important characteristic property of PHBV is its microbial degradability in natural ecosystem such as soil and compost^{34, 134, 135}. Figure.17 shows the weight loss curves of pure PHBV and PHBV-g-PVP samples with various extent of grafting (% G=17, 38 and 45) as a function of immersion in compost soil. It was found that the weight loss value of pure PHBV is about 28% before treatment, whereas the weight loss value for PHBV graft samples, irrespective of degree of grafting, is much higher than that of the pure PHBV. With the increase in graft degree, the weight loss value gradually increases, indicating that the biodegradability of PHBV can be enhanced by the grafting with PVP. This might be attributed to the increase of bulk hydrophilicity of the hydrophobic PHBV host polymers. Another possible reason for the high degradation of the investigated PHBV-g-PVP copolymer may be due to the differences in surface morphology. Visual examination of the undegraded surfaces of PHBV and PHBV-g-PVP copolymers showed that the surface of the PHBV graft samples had some roughness and possessed some cracks compared with the surface of the base polymers. Thus the increase of the surface area, due to the roughness and cracks, is expected to increase the biodegradability of the film in soil by inducing the attachments of the environmental microbes. This finding is consistent with the studies conducted by Molitoris et al¹³⁶. Tsuji and Suzuyoshi¹³⁷ reported that the rate of hydrolysis of polyester was dependent on the surface area of the polymer exposed to hydrolysis.

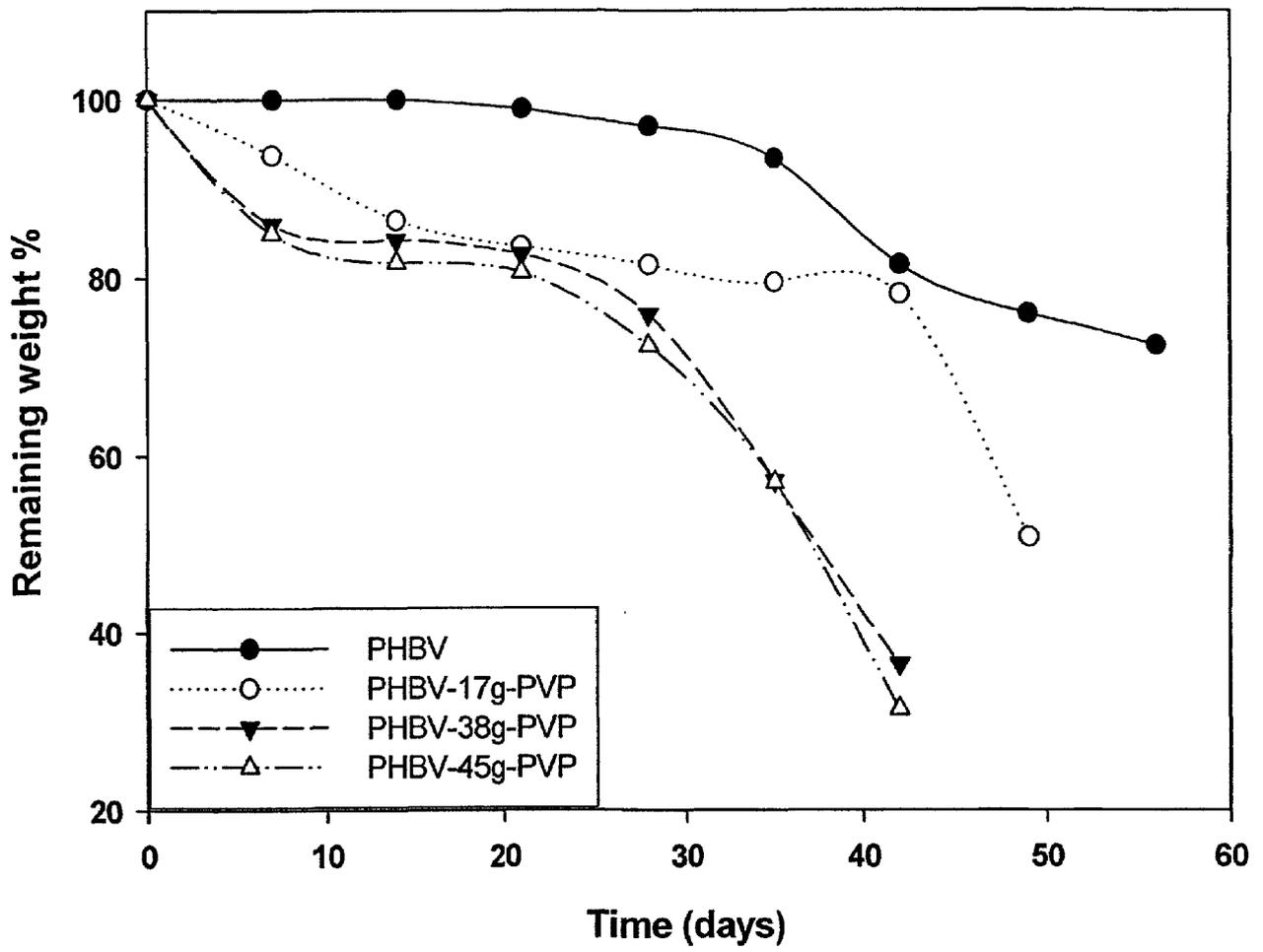


Figure 17: Biodegradation of PHBV and some grafted copolymers in active garden soil

3.2. Graft Copolymerization of Isopropylacrylamide (PIPA) onto poly (3-hydroxy butyrate-co-3-hydroxyvalerate)

3.2.1. Evidence of grafting of PIPA onto PHBV

In the study of the infrared spectra of PHBV, PIPA and some of grafted PHBV copolymers, shown in Fig.18, we found that, for PHBV, -as I said before for the first part of work- the carbonyl vibration consists of a peak centered at 1723 cm^{-1} and a shoulder at 1740 cm^{-1} , which are assigned to the vibrations of crystalline and amorphous carbonyl ester groups of the repeated units, respectively¹²⁶. For all the graft copolymers by IPA monomer a new absorption band was found in the region $1643\text{-}1647\text{ cm}^{-1}$ which is assigned to amide C=O which occurs at lower frequencies than the “normal” carbonyl absorption due to the resonance effect (amide I), and its intensity increases with increasing the grafting percentage . The band appearing at $1440\text{-}1200\text{ cm}^{-1}$ region is assigned to C-N stretching. This band is however to a large extent overlaid by the C-H and $\text{CH}_2\text{-}$ and $\text{CH}_3\text{-}$ bands in this region stemming from the main chains of the PHBV and PIPA graft chain. Moreover the bands due to stretching vibration of the CH_3 groups around $2900\text{-}2975\text{ cm}^{-1}$ are found to increase in intensity with the increase of the grafting extent.

$^1\text{H-NMR}$ spectra of the PHBV and the representative examples grafted PHBV-31g-PIPA with their assignments peak showing in Fig. 19. The spectrum of pure PHBV compared with that of the graft copolymer, I found that the most striking characteristics in the spectrum of the graft sample denoted by [a], [d], for the methyl groups appearing at 1.26 and 1.14 ppm. [g] is donated for the peak at 1.4 ppm for the methylene group, and the peak at 4 ppm assigned to-the methine group denoted by [f], [h].

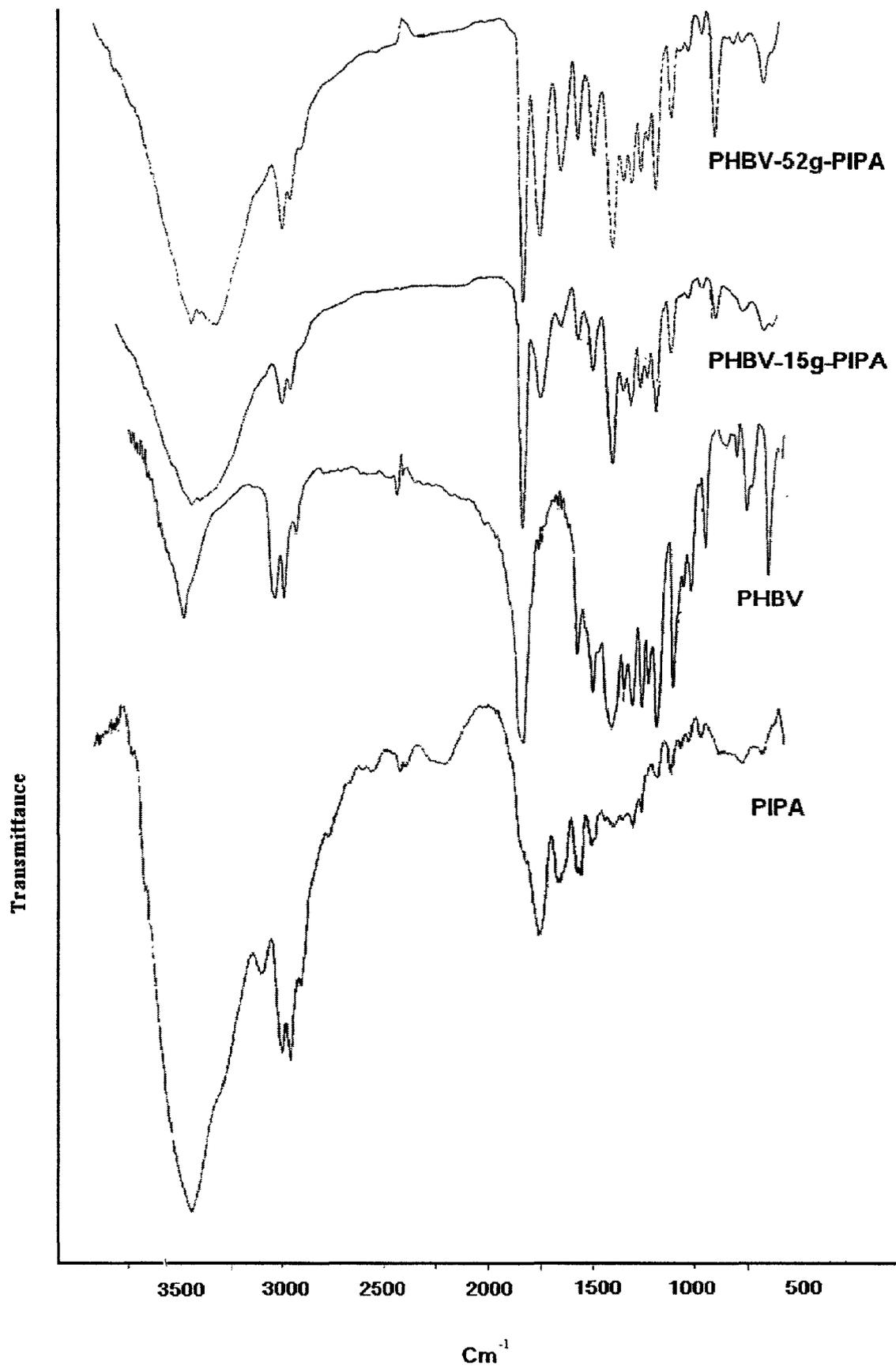


Figure 18: FTIR spectra of PHBV, PIPA, and graft copolymers (15, 52% G)

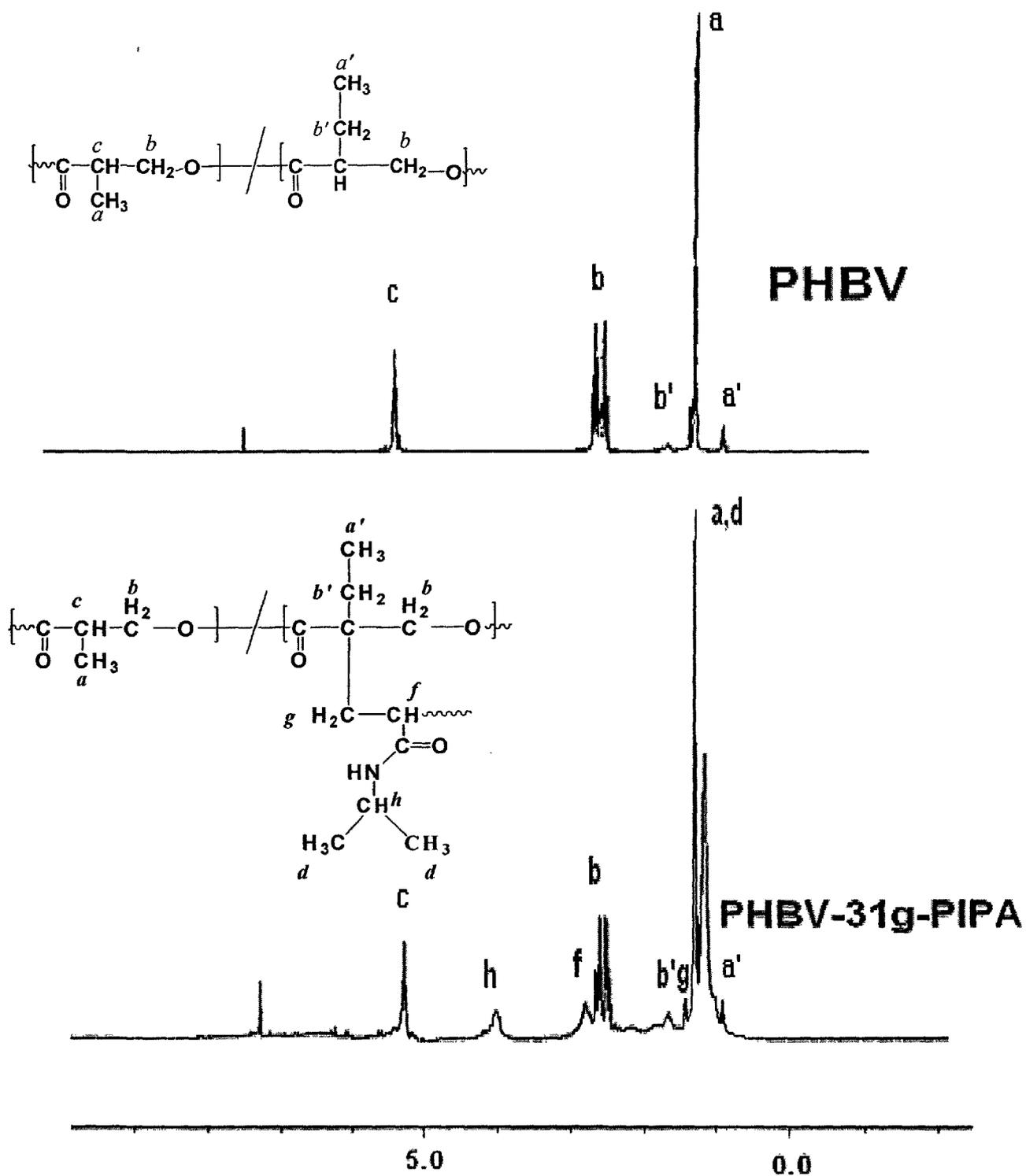


Figure 19: ¹H-NMR spectra of PHBV and PHBV-22 g-PVP graft sample

3.2.2. Influence of reaction conditions on the extent of grafting

As we studied in the previous system, the grafting of N-Isopropylacrylamid (IPA) onto PHBV in homogeneous medium was optimized by changing the initial concentration of monomer, initiator, and reaction time. The solvent chosen for this system is chlorobenzene because the grafting using methylene chloride as a solvent did not successfully achieve.

3.2.2.1. Effect of monomer concentration

The graft copolymerizations of IPA onto PHBV were carried out with the monomer concentrations ranged from 0.5 to 2 M. From Fig. 20, it can be seen that the grafting ratio was increased with increasing the molar concentration of IPA monomer to a maximum of 82 % up to 1 M, and thereafter they were rapidly decreased. The decreasing phenomena at higher monomer concentration mean that PIPA homopolymer is formed more easily than a graft polymer, (PHBV-g-PIPA).

3.2.2.2. Effect of initiator concentration

The effects of initiator concentration on the graft copolymerizations are shown in Fig. 21. The grafting ratio increased with increasing initiator concentration up to 8.0×10^{-3} M of BPO but thereafter they decreased rapidly with further increase of initiator concentration. The decrease in grafting is attributed to the reason that PIPA homopolymer was formed more readily than PHBV-g-PIPA at higher initiator concentrations. On the other hand, it reveals that the formation of active radical from the reaction between the initiator and IPA is easier than the initiator and PHBV.

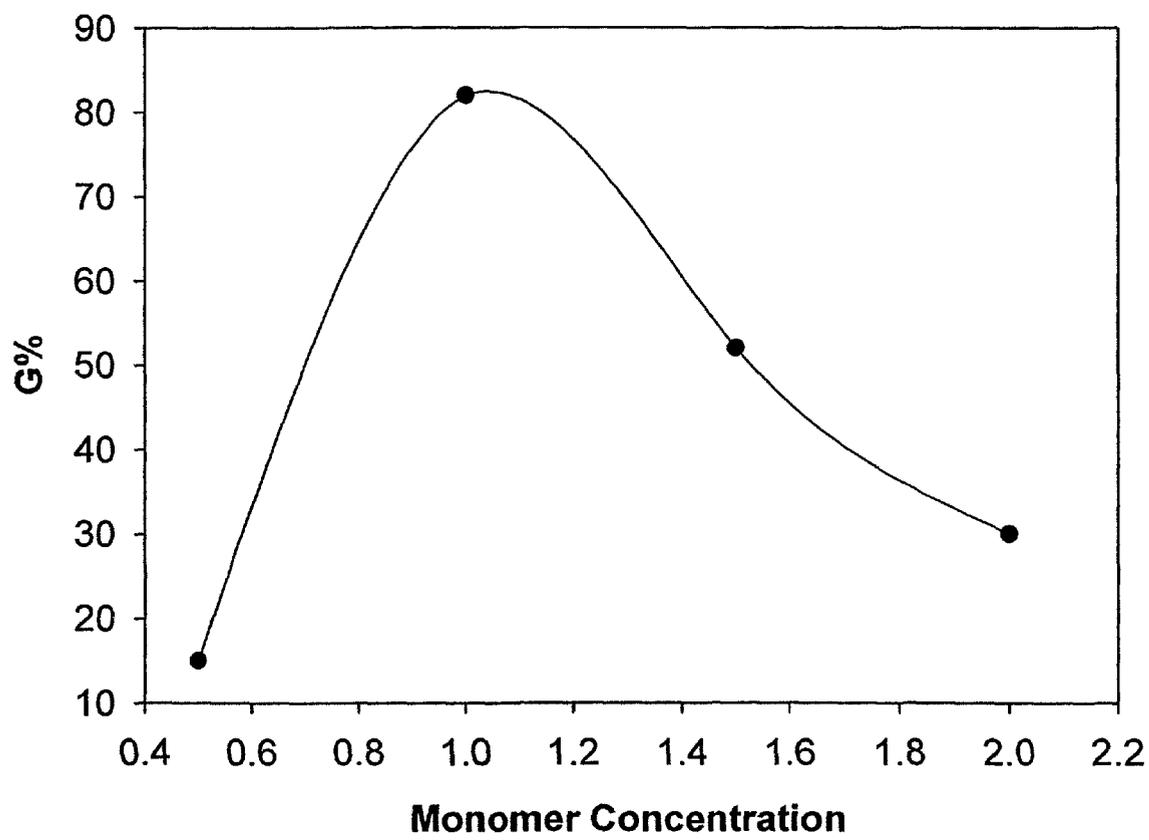


Figure 20: Effect of monomer concentration on the grafting reaction, $[I] = 8.0 \times 10^{-3} \text{ M}$

$T = 130^{\circ}\text{C}$

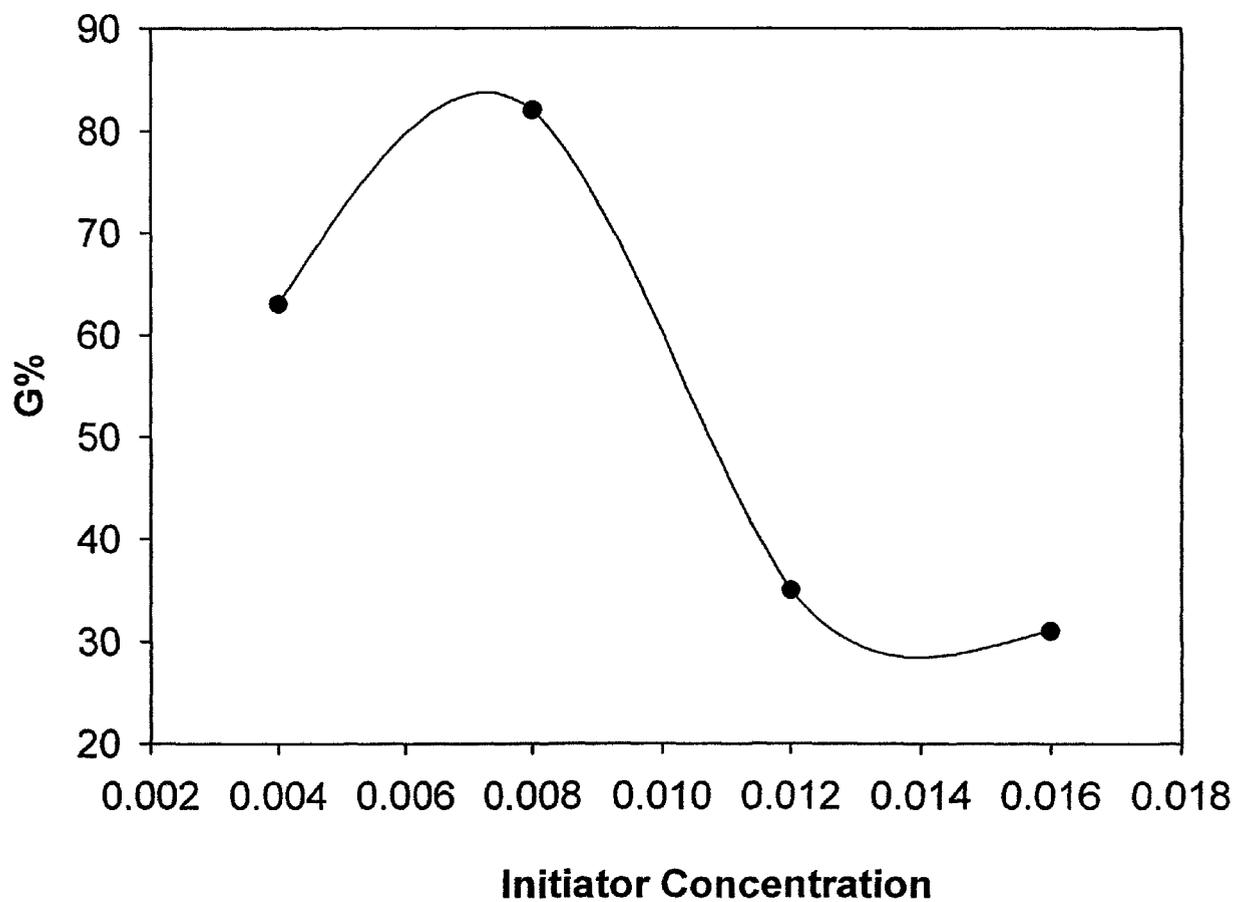


Figure 21: Effect of initiator concentration on the grafting reaction, $[M] = 1 \text{ M}$, $T = 130^\circ\text{C}$

3.2.2.3. Effect of reaction time

Reaction time is of great importance to graft copolymerization. The polymerization cannot be finished in a shorter period because the dissociation of BPO and the production of macroradicals must consume time, whereas the radicals would have lost their activity when the reaction time is too long. The effect of reaction time on graft degree is presented in Fig. 22. Graft degree gradually increases with reaction time, and begins to decrease after about 2 h, implying that graft copolymerization is almost finished. And the farther decreasing in the grafting may be due to degradation of the host polymer with the long time at the refluxing temperature.

3.2.3. Viscosity Measurements.

A similar trend of the viscosity of the grafted copolymers in the case of PVP has also been observed in the case of PIPA. The viscosity decreased with the extent of grafting as shown in Fig. 23 a similar explanation is valid here also

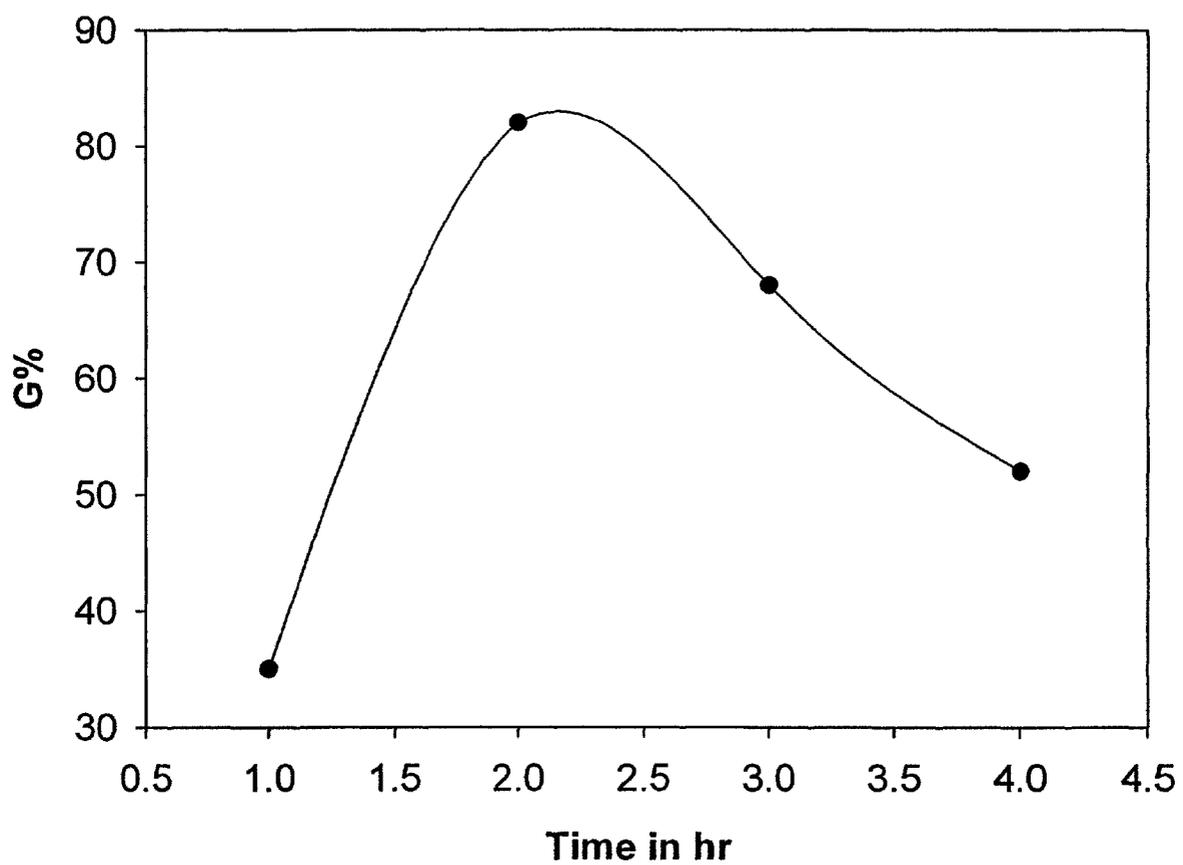


Figure 22: Effect of time on the grafting reaction $[M] = 1 \text{ M}$, $[I] = 8 \times 10^{-3} \text{ M}$, $T = 130^\circ\text{C}$

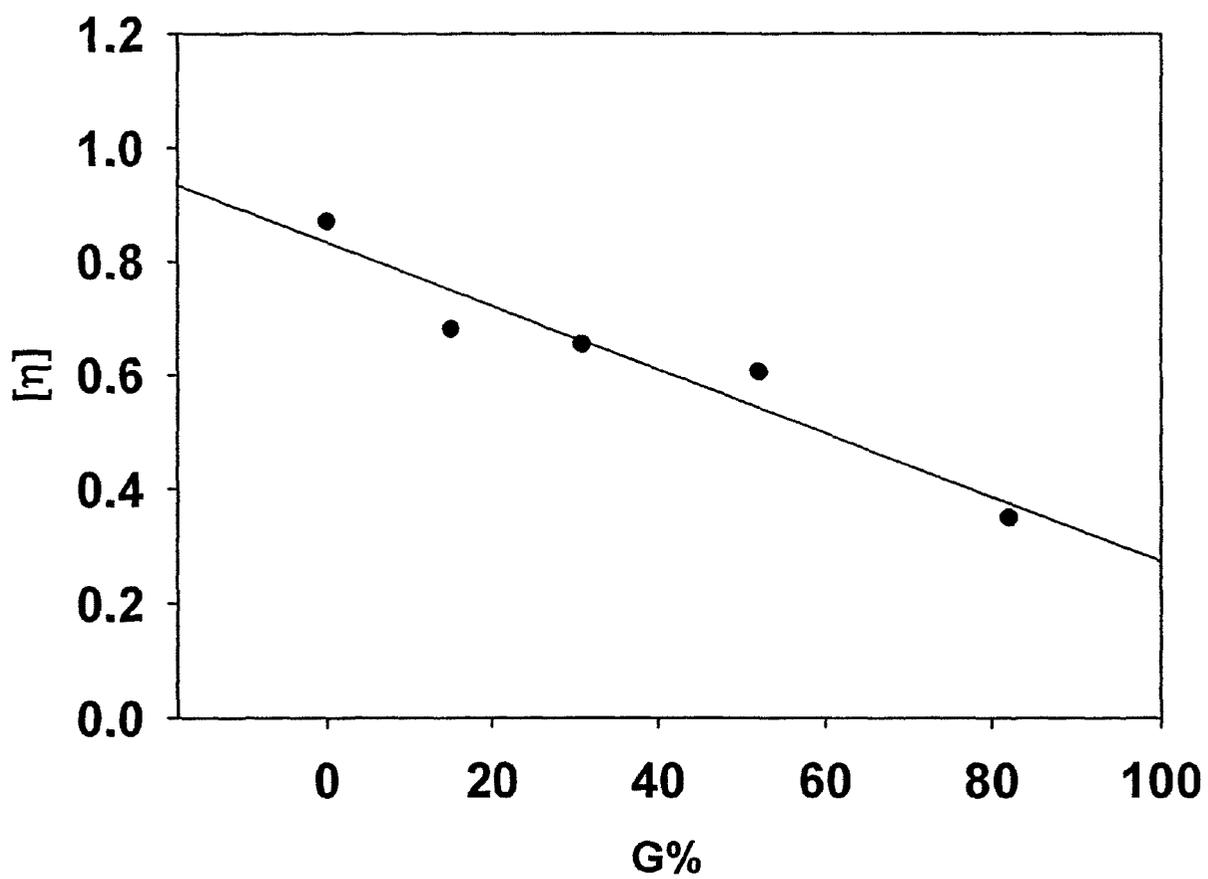


Figure 23: The effect of the grafting of IPA onto PHBV on the intrinsic viscosity

3.2.4. Thermal analysis

Fig.24 (a) shows the DSC curves of PHBV and some of the grafted copolymers recorded after quenching. The main thermal transitions of all the investigated samples are summarized in Table 6. The same behavior to that of the grafted copolymers in the case of PVP has also been observed in the case of PIPA. It was found that cold crystallization temperature (T_{cc}) values of the PHBV graft samples are shifted systematically to higher temperature with increasing the extent of grafting. This indicates that introduction of PIPA chains leads to a retardation of the crystallization of PHBV. The lower crystallization tendency is primarily due to the disturbance of the regular structure of the PHBV chains by the graft PIPA chains. From the data in Table 6, it has been found that the extent of grafting has no significant effect on the T_g values of the grafted samples which are almost constant. The original PHBV shows double melting peak. The peak appearing at higher temperature is assigned to the melting of the reorganized crystal formed during DSC heating process, while the lower temperature one, which appears as a shoulder, corresponds to the fusion of the crystallites formed on cooling from the melt¹²⁷. From Fig. 24(a) and Table 6 one can notice that the lower peak is affected by grafting while that the peak at higher temperature is almost constant. This could be a consequence of grafting since the side graft chains could interfere with the crystallization process. These observations are consistent with the data in the previous work⁸⁷. The melting enthalpy (ΔH_m) slightly decreased (Table 6) with the increase in the graft degree, which implies that after grafting with PIPA, the crystallinity of PHBV declines.

The DSC thermograms of the copolymers recorded after slow cooling from the melt and annealing for one week at room temperature are depicted in Fig. 24 (b).

Table 6 Thermal characteristics of PHBV and its graft copolymers with PIPA

Sample code	T _m (oC)	T _{g1} (oC)	T _{g2} (oC)	T _{cc} (oC)	ΔH _m (J/g)	X _c %
PHBV	158.4, 170.7	–	7.7	64.6	67	45.9
PHBV-10g-PIPA	156.94,170.1	54.2	10.4	66.9	57.7	43.9
PHBV-31g-PIPA	155.81,170.46	56.9	10.1	68.5	51.4	41.4
PHBV-52g-PIPA	149.14,171.05	57.6	9.4	71.6	27.5	39.2
PHBV-63g-PIPA	144.58,169.79	60.2	9.6	72.6	20.5	38.0
PHBV-82g-PIPA	139.55,166.56	58.9	9.5	73.1	9.4	35.8

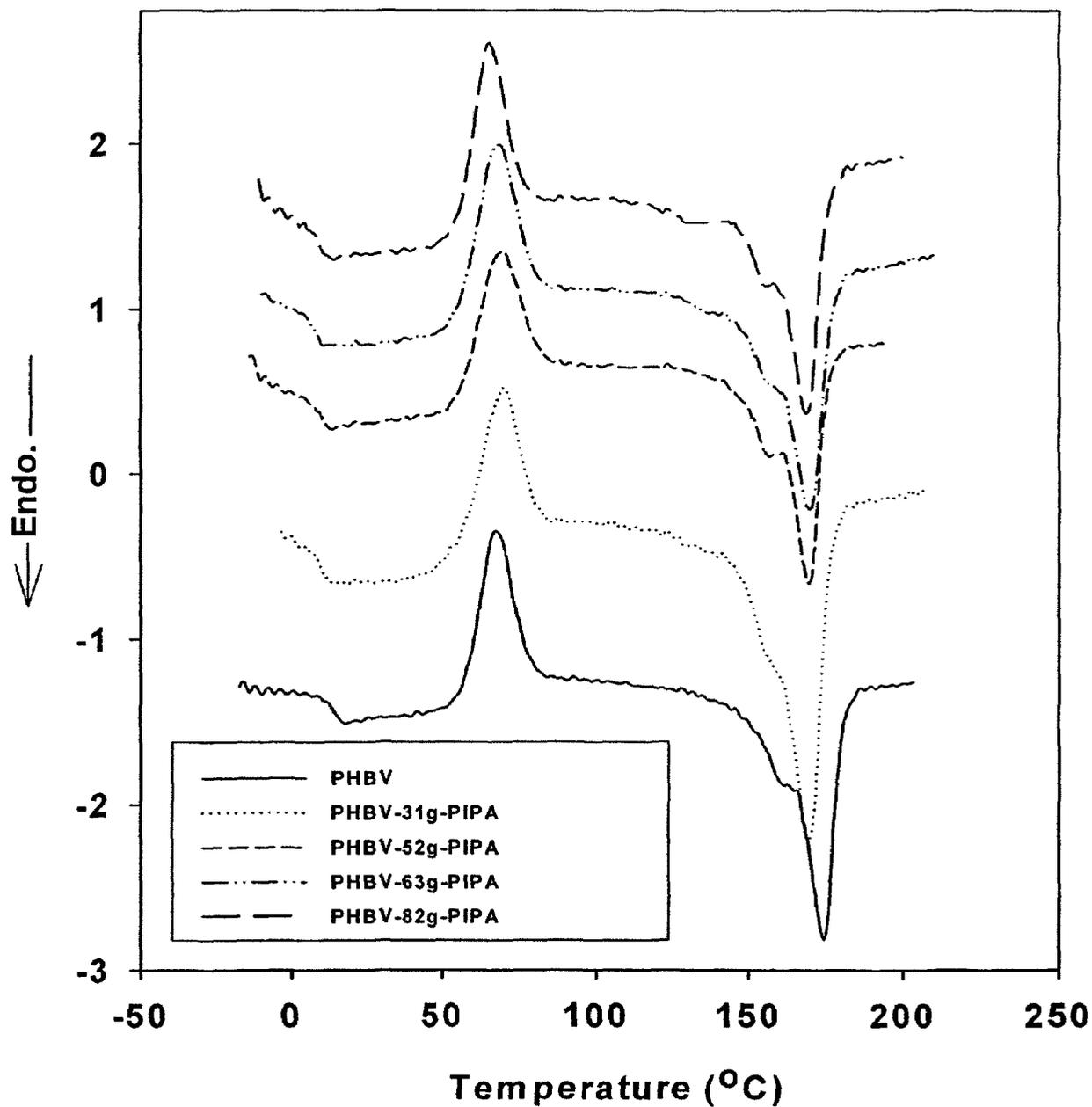


Figure 24(a): DSC curves of PHBV and some grafted copolymers recorded after quenching (run II)

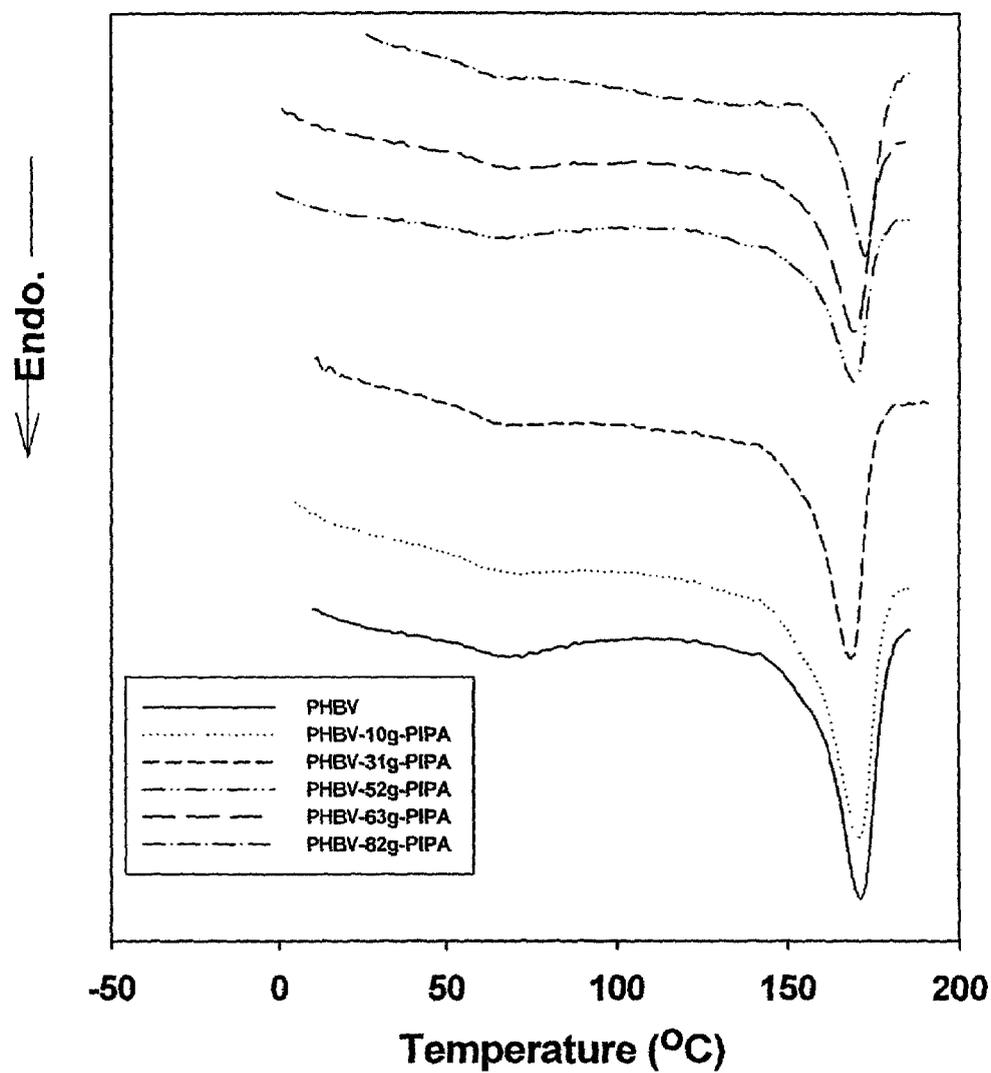


Figure 24(b): DSC curves of PHBV and some grafted copolymers recorded after slow cooling from melt and annealed for one week at room temperature (run III)

The results showed neither glass transition nor exothermic crystallization peaks corresponding to PHBV host polymer, indicating that slow cooling and annealing would allow complete crystallization of PHBV crystalline phase. The appearance of new T_g in the region from 50 to 60°C is probably related to amorphous mixed phase of PHBV base polymer and PIPA graft chains.

The normalized heat of fusion (melting enthalpy), i.e., the value calculated for 1 g of the PHBV, instead of 1 g of copolymer, is used to estimate the relative crystallinity percent (X_c %) of PHBV base polymer from equation(5).

The literature value being 146 J/g¹²⁸, W is the weight fraction of PHBV in the graft copolymer. Computed results are included in Table 6, and revealed that the degree of crystallinity slightly decreased by PIPA graft chains. This implies that the grafting of IPA monomer occurred mainly in the amorphous region of main chain polymer.

Fig. 25 illustrates the TGA and DTGA of the PHBV-30g-PIPA and PHB-82g-PIPA copolymers. The thermogram of pure PHBV is included in the figure for the sake of comparison. In contrast to PHBV copolymer, whose thermal degradation takes place as a single step in the temperature range 260 to 350°C¹²⁹, the decomposition of the grafted PHBV polymers is more complex.

The decomposition of the graft copolymers takes place by two steps in addition to water loss around 110°C. The main decomposition temperature takes place around 285°C followed by one smaller peak at around 390°C depending on the extent of grafting. The first peak is probably due to PHBV main chain decomposition, while the other is due to PIPA graft chains. It can be seen that grafting of the host polymer did not affect much its thermal stability.

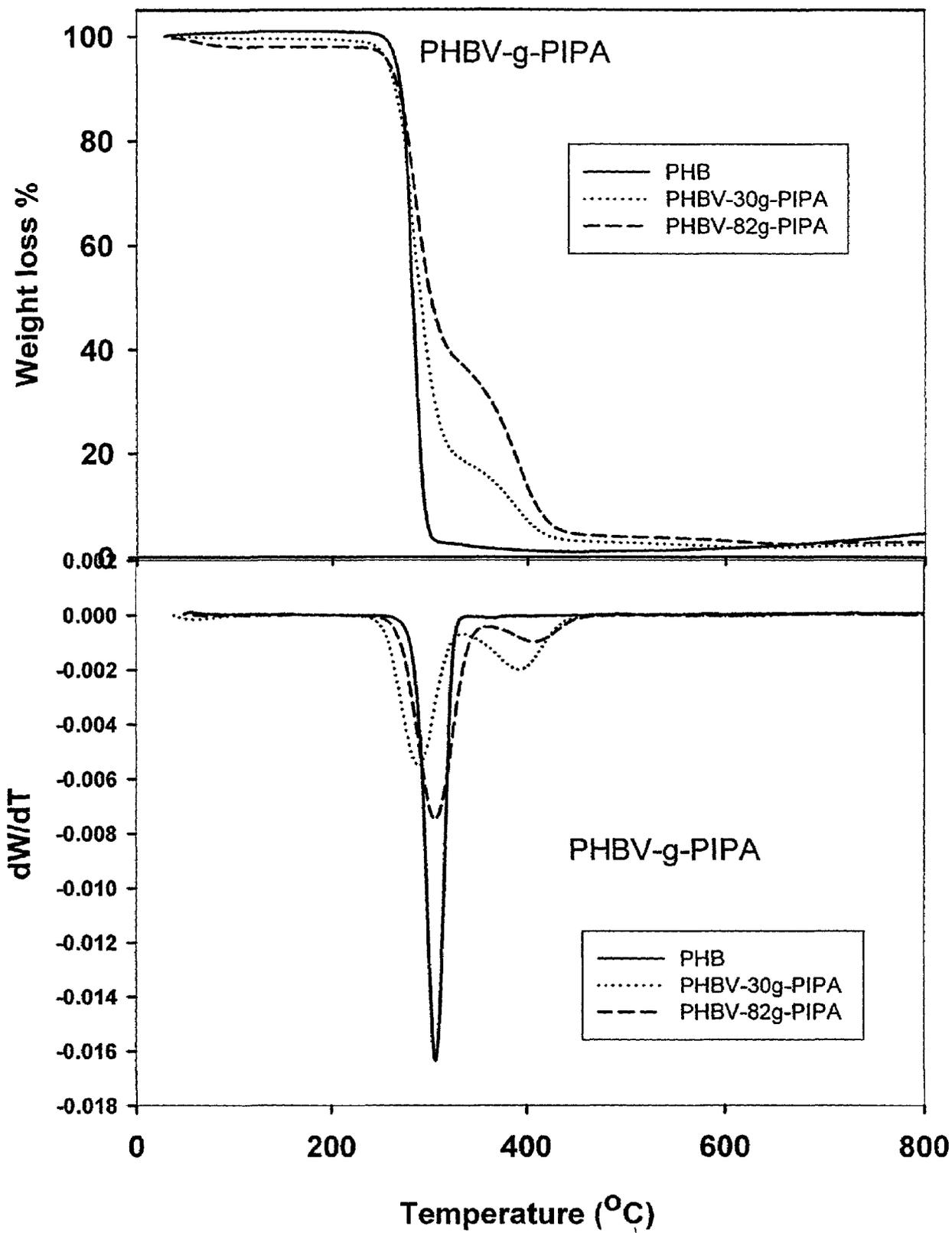


Figure 25: TGA and DTGA of PHBV and some grafted copolymers

3.2.5. Wide angle XRD analysis

Fig. 26 shows the X-ray diffraction patterns of PHBV and PHBV-g-PIPA samples. The diffraction patterns of PHBV consisted of two major crystalline peaks at $2\theta = 13.5^\circ$ of the (020) plane and 16.9° of the (110)^{25, 130}. Similar to the first system in case of PVP, the grafting with PIPA has no effect on the positions of these two peaks and the corresponding relative intensity ratios are not changed significantly even when the extent of grafting reached 52%, indicating that the crystalline structure of PHBV is not changed with grafting. This also confirms that PIPA graft chains occurred only in the non-crystalline regions of PHBV.

3.2.6. Swelling behavior

Polyesters are generally hydrophobic materials. However, grafting of PHBV with PIPA -water soluble and well-known thermosensitive polymer- is expected to enhance the swelling ability of the final graft copolymers. Therefore, this section aims to investigate the swelling behavior of the graft products in different buffer solutions and at different temperatures. The effect of grafting percentage on the capacity of swelling is also studied.

3.2.6.1. Effect of pH

Fig. 27 shows the dependence of the swelling degree as a function of time at pH = 6.5 and 9 for PHBV-63g-PIPA sample as representative example. The degree of swelling gradually increases with time and levels off after 70 min, irrespective of pH value. In addition, increasing pH from 6.5 to 9 increases the equilibrium swelling capacity from ~120 to ~140 %.

3.2.6.2. Temperature Effect

It is well known that the PIPA is a smart thermosensitive material^{117, 118}. Fig. 28 shows the dependence of swelling kinetics of PHBV-63g-PIPA graft copolymer as the function of temperature (18, 35 and 70°C) in distilled (pH = 6.5). It was found that the affinity of the grafted copolymer to uptake water increases by rising the temperature. This phenomenon is probably attributed to the presence of the thermosensitive PIPA graft chains on the hydrophobic PHBV host polymer. This behavior is in accordance with many previous experimental findings^{121-123, 138-140}.

3.2.6.3. Effect of Grafting Percentage

Fig. 29 shows the dependence of the swelling degree at equilibrium as a function of the extent of grafting. It can be seen that pure PHBV almost has no affinity for water. Sample with higher grafting shows enhanced swelling compared with the sample of low grafting.

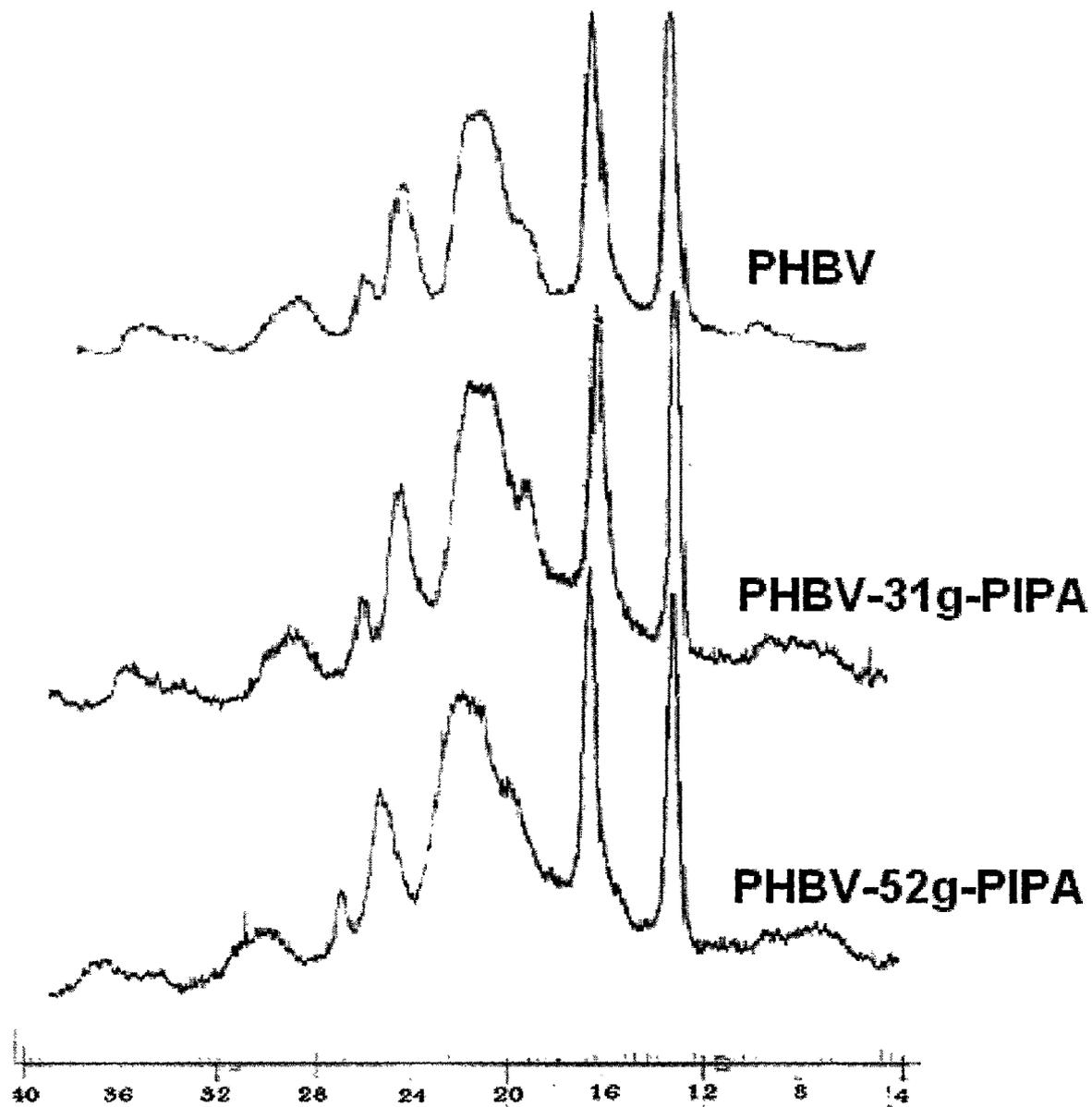


Figure 26: Wide-angle X-ray curves for of PHBV and some grafted copolymers

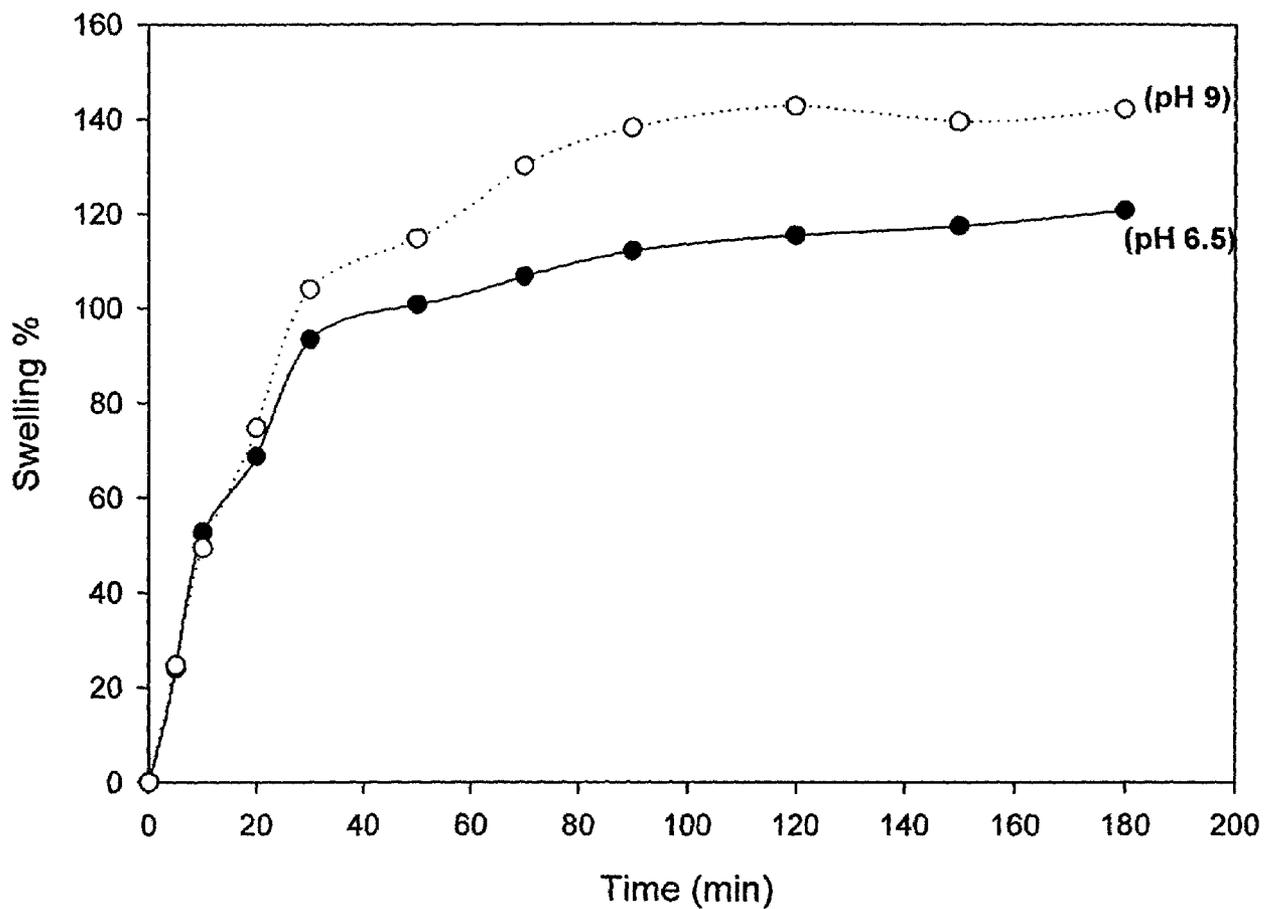


Figure 27: Swelling of PHBV- 63 g-PIPA copolymer as a function of time in distilled water, at different (pH = 6.5, 9) and at temperature = 25°C

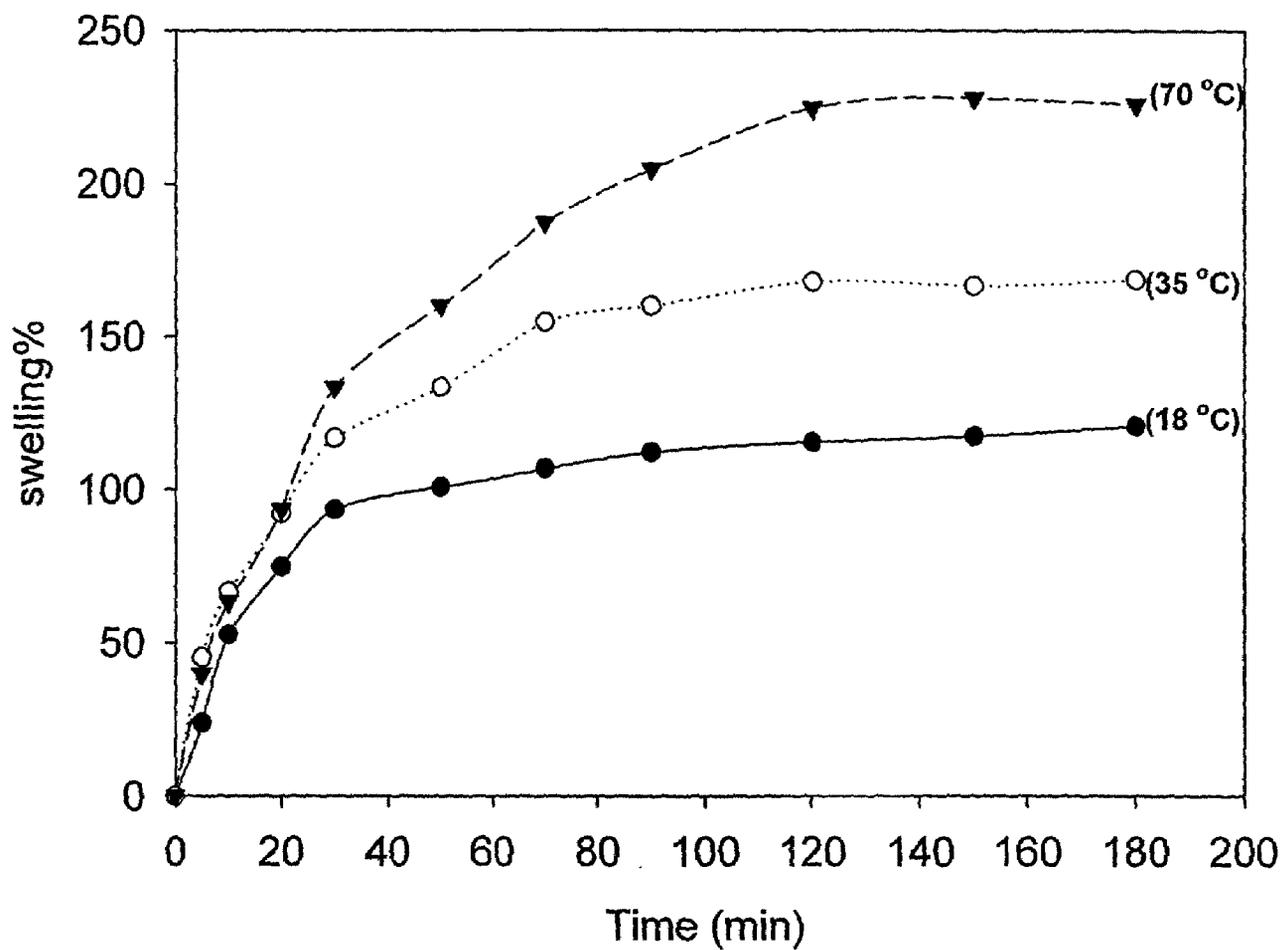


Figure 28: Swelling of PHBV- 63 g-PIPA copolymer as a function of time in distilled water, at different temperatures (18, 35, and 70 °C) and at constant pH = 6.5,

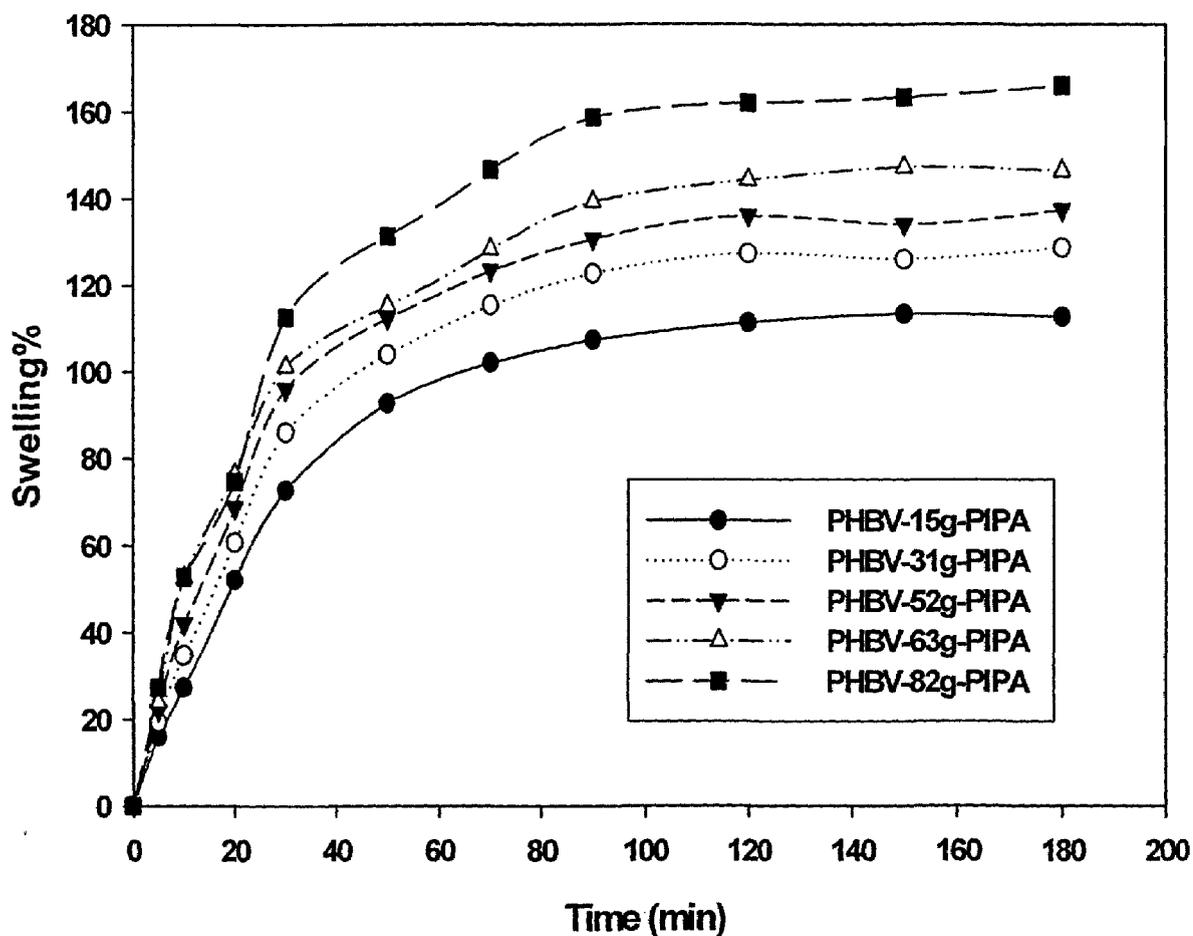


Figure 29: Swelling of PHBV-g-PIPA polymers at different grafting percent ($G\%=15, 31, 52, 63,$ and 82) as a function of time in distilled water, ($\text{pH} = 6.5$) and at temperature = 30°C

3.2.7. Biodegradability of PHBV and PHBV-g-PIPA

Fig.30 shows the weight loss curves of pure PHBV and PHBV-g-PIPA samples with various extent of grafting (% G=15, 31 and 63) as a function of incubation in composite soil.^{34, 134, 135} As I found in first part of work, the weight loss value of pure PHBV is about 28% before treatment, and by comparison, the weight loss value for PHBV graft samples is much higher than that of the pure PHBV. The figure shows that the increase in graft degree, leads to gradual increase in weight loss value, indicating that the biodegradability of PHBV can be enhanced by the grafting with PIPA. This might be attributed to the increase of bulk hydrophilicity of the hydrophobic PHBV host polymers. Another possible reason for the high degradation of the investigated PHBV-g-PIPA copolymer may be due to the differences in surface morphology. A similar morphology of the grafted copolymers in the case of PVP has also been observed in the case of PIPA, so the undegraded surfaces of PHBV and PHBV-g-PIPA copolymers showed that the surface of the PHBV graft samples had some roughness and possessed some cracks compared with the surface of the copolymers. Thus the increase of the surface area, due to the roughness and cracks, is expected to increase the biodegradability of the film in soil by inducing the attachments of the environmental microbes^{136, 137}. It has been reported that the rate of hydrolysis of polyester was dependent on the surface area of the polymer exposed to hydrolysis.

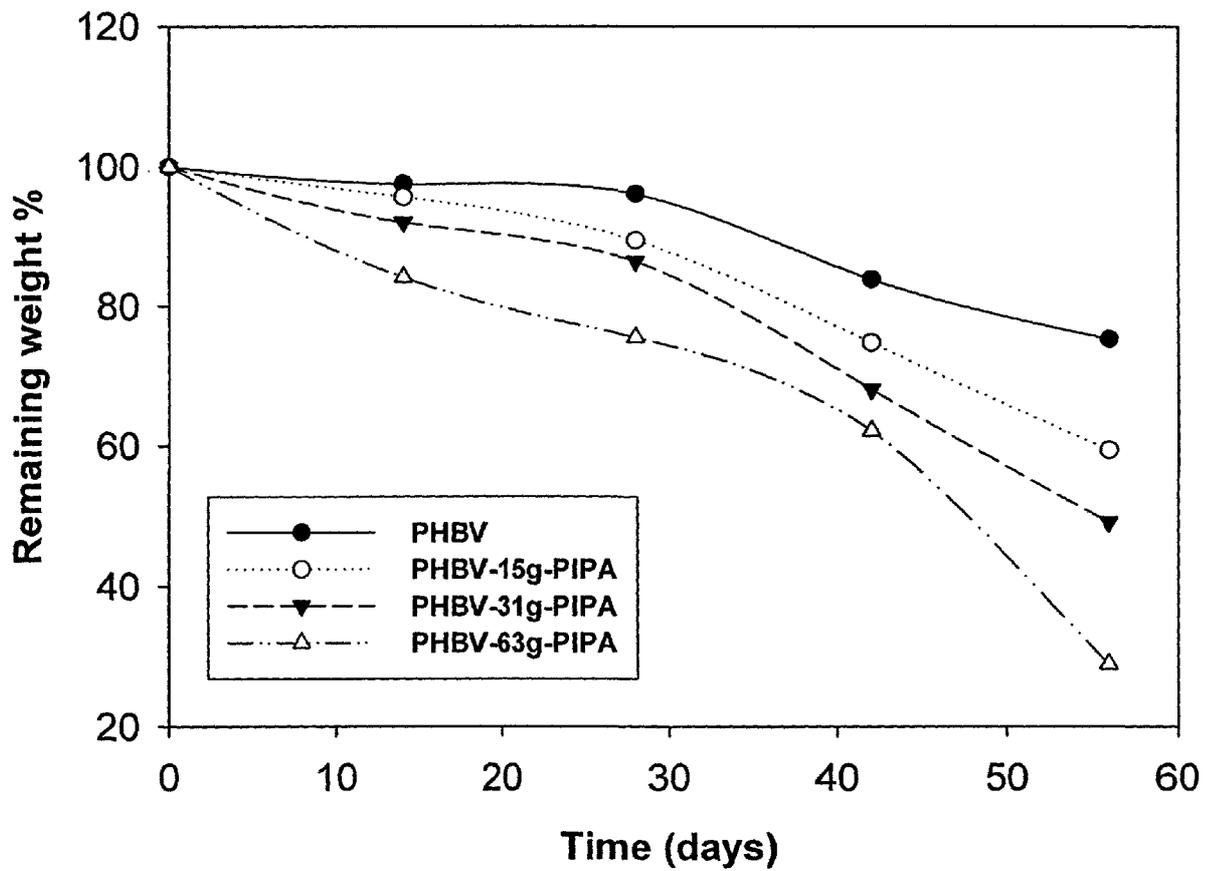


Figure 30: Biodegradation of PHBV and some grafted copolymers in active garden soil