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Research Article

Graft Copolymerization of Glycidyl Methacrylate/ Acrylamide Monomer Mixture onto Polyethylene Terephthalate Fibers; Characterization and Investigation of Some Properties

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Abstract

Polymers are highly important materials that are widely used in every aspect of our lives. Through grafting, polymers can be modified with desired monomers to acquire specific properties. Polyethylene terephthalate (PET) fibers possess many favorable characteristics such as cheap raw materials, low production costs, and high resistance to environmental effects. However, they also have disadvantages, such as limited water absorption capacity and dyeability due to their hydrophobic nature. This study aims to improve these negative aspects of PET using the graft copolymerization method. In the study, GMA (glycidyl methacrylate) and AAm (acrylamide) monomers containing different functional groups were grafted onto PET fibers using benzoyl peroxide (BPO) as the initiator. Additionally, lipase enzyme was immobilized on the grafted PET fibers, and the use of this immobilized enzyme in the hydrolysis of various types of oils was investigated. Ungrafted and grafted PET fibers were characterized using Scanning Electron Microscopy (SEM), Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), and Fourier Transform Infrared Spectroscopy (FTIR) to investigate the effects of various parameters on the grafting process. The water retention and dyeability properties of the grafted PET fibers were also demonstrated.

Keywords

"PET, GMA, AAm, Graft Copolymerization, Immobilization, Lipase"

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1. Introduction

Natural and synthetic polymers are used in many areas of our lives, such as medicine, nutrition, communication, transportation, and clothing. These polymers can be found in various forms, including fibers (Saeed, 2024), gels (Priyan, 2024), spheres (Lee ve Patel, 2022), nanoparticles (Rezaei, 2023.

Polymers with desired properties can be synthesized by graft copolymerization, which involves grafting monomers containing various functional groups onto polymers. Polymers obtained by this method are biodegradable and can be used in many fields, including agriculture, textiles, the paper industry, medical treatments, and the petroleum industry (Kumar vd., 2017). Industrially, PET is a condensation polymer obtained by the polymerization of ethylene glycol with terephthalic acid or dimethyl terephthalate (Siddiqui vd., 2021). PET not only possesses excellent mechanical properties and stability but also demonstrates resistance to acids, bases, and many chemicals, while being a transparent polymer (Askar vd., 2023). However, alongside these superior properties, PET has disadvantages such as low water absorption capacity and difficulty in dyeing (Rathod vd., 2021). To address these drawbacks, the grafting of suitable monomers onto PET fibers using the graft copolymerization method can improve the properties of the fibers, making them more suitable for enzyme immobilization and various bioengineering applications (Taştan vd., 2023).

GMA, which has a wide range of applications in polymer chemistry, is a monomer containing methacrylic acid and glycidyl functional groups. GMA is frequently preferred in the production of polyester resins and many industrial applications (Dobrovolsky vd., 2016). The presence of methacrylate and epoxide groups increases the chemical reactivity of GMA.(Mashabi vd., 2022). Thanks to these properties, GMA has become a preferred monomer in adhesives, coatings, and certain biomedical applications.

AAm, an amide derivative of acrylic acid, enables various chemical reactions due to its functional groups (Stadler ve Gökmen, 2024). AAm is a monomer used in numerous industrial applications, including water treatment processes, the textile and paper industries, and certain biomedical fields such as contact lens production (Zamani vd., 2017).

Immobilization forms a system that supports the enzyme through the matrix to which it is bound and the method of binding. This binding can occur through various methods, such as adsorption, ionic, or covalent bonding. Enzyme immobilization is a technique that preserves the catalytic activity of the enzyme and allows its repeated use (Khan, 2021).

Lipase is an important enzyme involved in fat metabolism. It catalyzes the hydrolysis of fats and oils into fatty acids and glycerol (Adia, 2024).

In the literature, studies have been encountered where various monomers or monomer mixtures were grafted onto PET fibers; however, no studies were found in which the GMA/AAm monomer mixture was grafted together onto PET fibers. This study aims to improve the aforementioned drawbacks of PET fibers by introducing the functional groups of these monomers into the PET fibers, thereby enabling the use of grafted fibers in various biotechnological applications. The grafted PET fibers were modified with ethylenediamine (EDA), and lipase enzyme was immobilized onto these modified fibers using the covalent binding method. In this study, which investigated the effects of certain parameters on immobilization, the impact of immobilized lipase enzyme on the hydrolysis of various types of fats was also examined.

Table 1. Symbols and Abbreviations

PET	Polyethylene terephthalate
GMA	Glycidyl methacrylate
AAm	Acrylamide
BPO	Benzoyl peroxide
SEM	Scanning Electron Microscopy
DSC	Differential Scanning Calorimetry
FTIR	Fourier Transform Infrared Spectroscopy
TGA	Thermogravimetric Analysis
EDA	Ethylenediamine

2. Materials and Methods

2.1. Chemical Substances

PET fibers were obtained from SASA (Sun'i ve Sentetik Elyaf A.Ş). The fibers were washed in acetone for several hours using a Soxhlet apparatus, then dried and used. GMA and AAm were purchased from Aldrich and used as monomers in this study. The monomers were used as received. BPO, obtained from Merck, was recrystallized in a mixture of methanol and chloroform and used as an initiator in the polymerization process. The lipase enzyme derived from Candida rugosa was purchased from Sigma. Other chemicals used in the experiments are of analytical grade.

2.2. Graft Copolymerization Method

PET fibers were placed into a polymerization tube of a specific volume, and an appropriate amount of GMA/AAm monomer mixture was added. Subsequently, 2 mL of BPO dissolved in acetone was added to the monomer mixture, and the volume was completed to 20 mL with distilled water. The tube was then immersed in a temperature-controlled water bath (Stuart SBS40). Fibers were removed from the medium at specific intervals and washed with acetone and distilled water. After drying the grafted fibers, the grafting percentages were calculated. The grafting percentage (%G) was determined using the masses of the grafted and original PET fibers, based on the formula below (Çelik, 2004).

$$\% A = \frac{m_g - m_o}{m_o} \times 100 \quad (1)$$

m_g: mass of the grafted PET fiber
m_o: mass of the original PET fiber

2.3. Determination of AAm Content in Grafted PET Fibers

The percentage of AAm incorporated into the structure of PET fibers grafted with the GMA/AAm monomer mixture was calculated using the nitrogen content determined by elemental analysis, based on the formula below (Coşkun, 2008).

$$\% (AAm) = \% N \left(\frac{W_g}{W_o \times 14}\right) \times M_{AAm} \quad (2)$$

M_{AAm}: Molecular weight of AAm (71,08 g/mol)
W_g: mass of the grafted PET fiber
W_o: mass of the ungrafted PET fiber

The grafting percentage of GMA was determined by subtracting the grafting percentage of AAm from the total grafting percentage grafted onto the PET fibers.

2.4. Determination of the Water Absorption Capacity of Grafted PET Fibers

PET fibers with different grafting percentages were immersed in pure water at 25°C for a specific period. The PET fiber samples were then removed from the medium, blotted with filter paper, and weighed. The water absorption capacity of the grafted PET fibers was calculated using the equation below (Yamada vd., 2023)

$$\% \text{ Water Absorption Capacity} = \frac{m_{s-} m_k}{m_k} \times 100 \quad (3)$$

ms: Mass of fibers soaked in pure water (g)
mk: Mass of dried fibers (g)

2.5. Experiments on Dyeing of PET Fibers

To demonstrate the dyeing properties of GMA/AAm grafted PET fibers with different grafting percentages, the fibers were mixed under specific conditions in dye solutions with a concentration of 50 mg/L of acidic Congo red and basic methylene blue dyes. The dyeing properties of PET fibers with Congo red were evaluated under the conditions specified by Bozkaya et al., while those with methylene blue were evaluated under the conditions specified by Arslan et al. The dye concentrations in the solutions were calculated using pre-prepared standard calibration curves. Measurements were taken with a TU-1810 DASPV model spectrophotometer at wavelengths of 497 nm and 665 nm for Congo red and methylene blue, respectively (Bozkaya vd., 2021; Arslan vd., 2019).

2.6. Preparation of Enzyme Support Material

The lipase enzyme was immobilized onto GMA/AAm grafted PET fibers using the covalent bonding method. For use as support material in lipase enzyme immobilization, GMA/AAm grafted PET fibers were:

Method 1: The modified PET fibers, obtained by mixing in EDA for a specific period, were activated with glutaraldehyde, and then the lipase enzyme was immobilized (Temoçin, 2013).

Method 2: The modified PET fibers synthesized via the Hofmann rearrangement reaction were activated with glutaraldehyde, and then the lipase enzyme was immobilized (Temoçin, 2009).

For the Hofmann rearrangement reaction, PET fibers grafted with a GMA/AAm monomer mixture (0.03 g) were mixed in Erlenmeyer flasks containing NaOH and NaOCl solutions of specified volumes and concentrations at 20°C with a shaking speed of 100 rpm. The fibers removed from the medium were washed several times with buffer solution and pure water. To determine the optimal reaction conditions, the effects of NaOH and NaOCl concentrations, as well as mixing time, on the Hofmann reaction were investigated. Additionally, the activation time of the modified PET fiber in glutaraldehyde and the mixing times in the enzyme solution were also optimized.

Method 3: PET fibers modified using both of the above methods were activated with glutaraldehyde, and then the lipase enzyme was immobilized. The study continued with the method that showed the highest enzyme activity among the methods used.

2.7. Enzyme Immobilization and Optimization of Immobilization Conditions

Modified PET fibers were activated by mixing with a 5 mL glutaraldehyde solution at a concentration of 4% (w/w) at 150 rpm for a specified period. To remove unreacted glutaraldehyde from the PET fibers, they were washed with a buffer solution (pH = 7) and deionized water. The immobilization of lipase enzyme onto the glutaraldehyde-activated modified fibers was achieved by mixing the fibers with vials containing lipase enzyme solution at specific concentrations and volumes for a certain period.

The effect of solution pH and the grafting percentage of PET fibers on the immobilization of the lipase enzyme onto the support material was investigated. The impact of solution pH on lipase enzyme immobilization was examined within the pH range of 3-8, and enzyme activity was determined. The pH value at which maximum activity was achieved was considered the optimal pH value for immobilization.

To investigate the effect of grafting percentage on enzyme immobilization, lipase enzyme was immobilized onto PET fibers with specific grafting percentages. The PET fibers that achieved maximum enzyme activity were considered the fibers with the optimal grafting percentage, and the study was continued using fibers with this grafting percentage.

2.8. Determination of Lipase Enzyme Activity

Olive oil was used as the substrate to determine the activity of the lipase enzyme. Olive oil was dissolved in iso-octane to create an organic phase. Lipase activity experiments were conducted in a biphasic reaction medium formed by this organic phase and a buffer solution. Free (1 mg/mL, 0.1 mL) and immobilized lipase enzymes were incubated in a biphasic hydrolysis medium consisting of 2 mL olive oil-iso-octane and buffer solutions. The incubation was carried out in a shaking water bath set to 35°C, with a shaking speed of 150 rpm for 30 minutes. The organic phase containing the hydrolysis product was transferred into a test tube and allowed to stand for a few minutes to separate the phases. Lipase activity experiments were repeated under the same conditions in the absence of free and immobilized enzymes, and the absorbance values obtained were considered as reference values. The activity of the lipase enzyme was determined by monitoring the change in the concentration of free fatty acids produced as a result of the hydrolysis of olive oil by the enzyme. The concentration of the released free fatty acids was determined by measuring the optical density of the colored solution formed by the interaction of fatty acids with Cu(II) ions in iso-octane. The measurements were conducted at a wavelength of 715 nm using a spectrophotometer (Yiğitoğlu, 2010).

The calibration curve was prepared using oleic acid-iso-octane solutions with concentrations in the range of 0.5–3 mg/mL. For each concentration, 2 mL of the solution was transferred into a test tube containing 0.5 mL of copper-pyridine reagent. The tubes were then mixed at a specific speed for one minute using a vortex mixer.

1 Unit (Enzyme Activity): Defined as the amount of lipase enzyme that releases 1 μmol of free fatty acid per minute. The % relative activity of the lipase enzyme was calculated using the following formula.

$$\text{Relative Activity (\%)} = \frac{\text{Activity}}{\text{Maximum Activity}} \text{ x } 100 \quad (4)$$

2.9. Effect of pH on Enzyme Activity

To investigate the effect of solution pH on lipase enzyme activity, a biphasic system was prepared consisting of olive oil dissolved in iso-octane at a concentration of 455 mg/mL and buffer solutions with pH values ranging from 3 to 9. The activities of free and immobilized lipase were determined by incubating the biphasic system in a water bath at 35°C with a shaking speed of 150 rpm for 30 minutes.

2.10. Effect of Temperature on Enzyme Activity

To investigate the effect of temperature on lipase enzyme activity, a biphasic system was prepared consisting of olive oil dissolved in iso-octane at a concentration of 455 mg/mL and a buffer solution. The activities of free and immobilized lipase were determined by incubating the biphasic system in a water bath at temperatures ranging from 20 to 70°C, with a shaking speed of 150 rpm, for 30 minutes.

2.11. FTIR Analysis of PET Fibers

The FTIR spectra of PET fiber samples were obtained using a Bruker Vertex 70 V model spectrometer by preparing KBr pellets.

2.12. Use of Lipase Enzyme in Oil Hydrolysis

Free and immobilized lipase enzymes were used in this study for the hydrolysis of different types of oils. In the hydrolysis experiments, PET fibers grafted with 122% GMA/AAm (70/30 mol) were used. After being modified with EDA and activated with glutaraldehyde, lipase enzyme was immobilized onto the monomer mixture-grafted PET fibers. The oils used in the hydrolysis experiments included sunflower oil, olive oil, canola oil, corn oil, and hazelnut oil. The activity for each type of oil was determined as described above.

3. Research Findings and Discussion

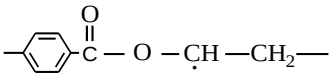
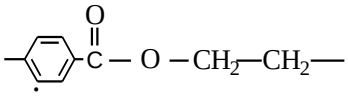
3.1. Grafting Mechanism

Different monomers can be grafted onto PET fibers using the graft copolymerization method. This process can be carried out with the aid of chain transfer reactions. Free radicals in the polymerization medium abstract a hydrogen atom from the PET fiber, forming active centers on the fiber. Monomers can then be grafted onto the fiber through these active centers. The corresponding mechanism is illustrated below. Additionally, the potential model for the grafting of the GMA/AAm monomer mixture onto the PET fiber is shown in Figure 1. It is assumed that the radicals below are formed as a result of the thermal decomposition of BPO.

$$C_6H_5COO - OOCH_5C_6 \rightarrow 2C_6H_5COO. \quad (5)$$

$$C_6H_5COO. \rightarrow C_6H_5. + CO_2 \quad (6)$$

The $C_6H_5COO\cdot$ and $C_6H_5\cdot$ radicals form active centers on PET in the polymerization medium (Saçak, 1991).

$R\cdot + PET \rightarrow PET\cdot$  and  (7)

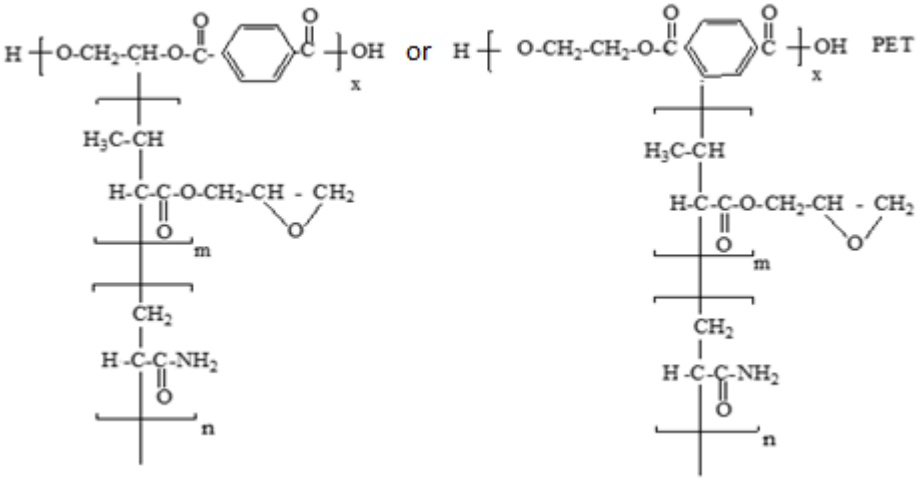


Figure 1. Possible model for PET fiber grafted with GMA/AAm monomer mixture

3.2. The Effect of Monomer Mixture Ratio on Grafting Percentage

GMA, AAm, and GMA/AAm monomer mixtures were grafted onto PET fibers, and the effect of the GMA/AAm monomer mixture ratio on the grafting percentage is shown in Figure 2. The monomer mixture concentration grafted onto the PET fibers was kept constant at 0.3 M, and the grafting percentages were determined by varying the amounts of GMA and AAm monomers.

When GMA is grafted onto PET fibers alone, the grafting percentage increases with the GMA concentration and reaches 141.63% at a concentration of 0.3 M, as shown in Figure 2. Similarly, when AAm is grafted onto PET fibers alone, the grafting percentage also increases with the AAm concentration, reaching 11.21%. The grafting percentages in the mixtures are higher than the individual grafting percentages of GMA and AAm. A significant increase in the grafting percentage was observed when GMA and AAm monomer mixtures were grafted onto PET fibers together. For example, the maximum grafting percentage was calculated as 177.75% at a 90/10 molar ratio of GMA to AAm. At the same mixture ratios, the grafting percentages of GMA and AAm when grafted individually are 123.42% and 2.69%, respectively. Similar results have been reported in studies on the grafting of maleic acid/methacrylamide (Coşkun ve Akdeniz, 2010) and 2-methylpropenoic acid (MPA)/acrylonitrile (AN) (Arslan ve Günay, 2017) monomer mixtures onto PET fibers.

The grafted PET polymer chains contain the functional groups of GMA and AAm monomers. The grafting percentage of AAm onto PET fibers was determined through elemental analysis based on the nitrogen content percentage in the fibers. The grafting percentage of GMA onto PET fibers was calculated by subtracting the grafting percentage of AAm from the total grafting percentage.

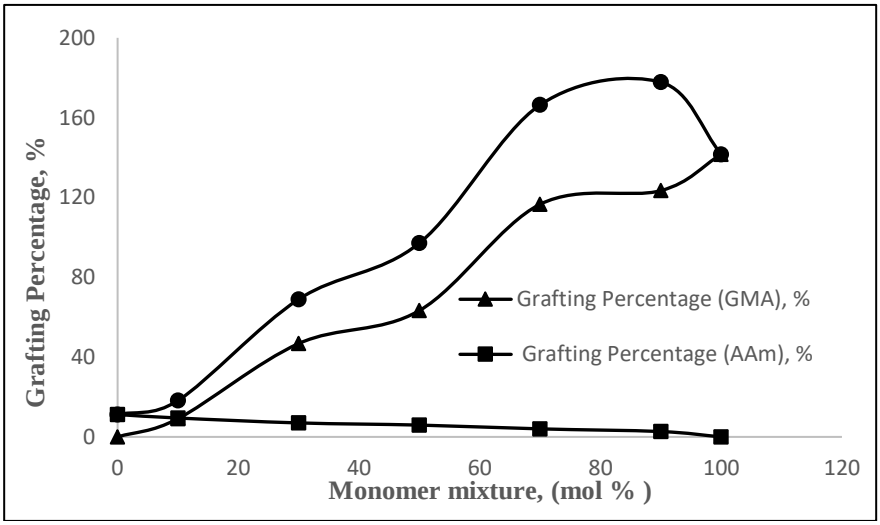


Figure 2. The Effect of GMA/AAm Monomer Mixture Ratio on Grafting Percentage
[GMA/AAm] = 0.3 M; [BPO] = 1.2×10^{-2} M; T = 80 °C; t = 180 min

As seen in Figure 2, the grafting percentage of AAm onto PET fibers in the presence of GMA shows a greater increase compared to its grafting percentage when used alone. This increase is believed to be due to a synergistic effect. The grafting percentages of monomers with low individual grafting efficiency can be enhanced through synergistic effects when grafted together with monomers that exhibit high grafting percentages (Deo, vd., 2008).

3.3. The Effect of Temperature on Grafting Percentage

The effect of temperature on the grafting of the GMA/AAm monomer mixture onto PET fibers was investigated within specific temperature ranges, and the results are presented in Figure 3. It was observed that the grafting percentage increased as the temperature was raised to 80 °C. The maximum grafting percentage for the GMA/AAm monomer mixture onto PET fibers was achieved at 80 °C. This temperature corresponds to the glass transition temperature (Tg) of PET. At temperatures above this Tg value, the polymer chains of PET gain mobility, facilitating the diffusion of monomer molecules into the PET fibers, which is thought to contribute to the increase in grafting percentage.

Additionally, it is believed that the decomposition rate of the BPO initiator increases with the rise in ambient temperature, leading to a higher radical concentration in the medium and, consequently, an increase in the grafting percentage (Yiğitoğlu ve Arslan, 2007).

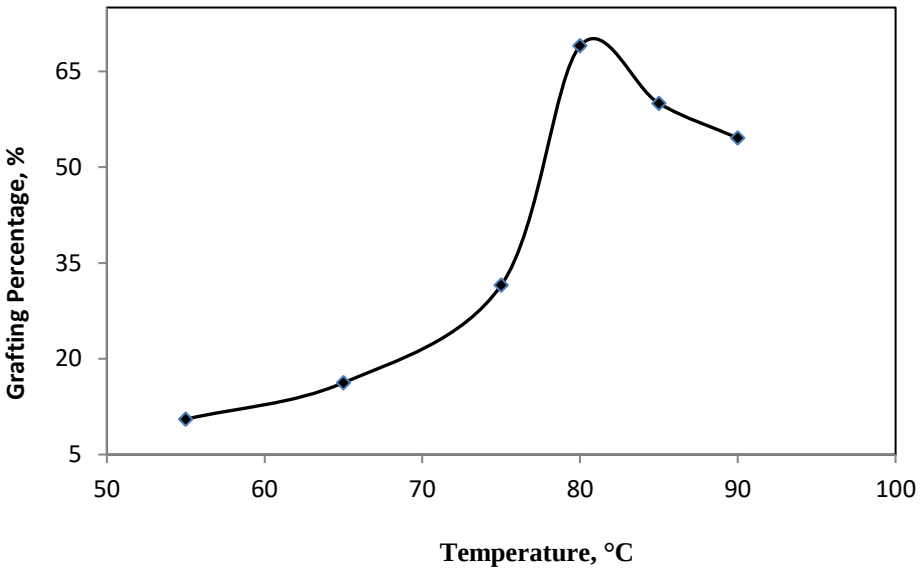


Figure 3. The Effect of Temperature on Grafting Percentage
[GMA/AAm] = 0.3 M; [BPO] = 1.2×10^{-2}; t = 240 min

In the grafting of the GMA/AAm monomer mixture onto PET fibers, a decrease in the grafting percentage was observed when the temperature exceeded 80 °C. It is thought that as the temperature increases, the termination reactions begin due to the combination of BPO radicals with each other, leading to a decrease in the grafting percentages. Similar results have also been observed in the grafting of the 4-VP/HEMA monomer mixture onto PET fibers (Yiğitoğlu ve Arslan, 2007).

3.4. The Effect of Polymerization Time on Grafting Percentage

The effect of polymerization time on the grafting of the GMA/AAm monomer mixture onto PET fibers was investigated at specific time intervals, and the results are shown in Figure 4. It was observed that the grafting percentage increased as the polymerization time increased. The maximum grafting percentage (74.52%) was achieved at 120 minutes, with no further increase observed at longer durations. The increase in grafting percentage up to 120 minutes can be attributed to the formation of new grafted chains on the PET fibers. After 120 minutes, the concentration of copolymers and homopolymers in the medium increased, leading to higher viscosity. This increased viscosity likely caused the formation of a diffusion barrier on the fibers, resulting in no further change in the grafting percentage (Azizinezhad, 2014).

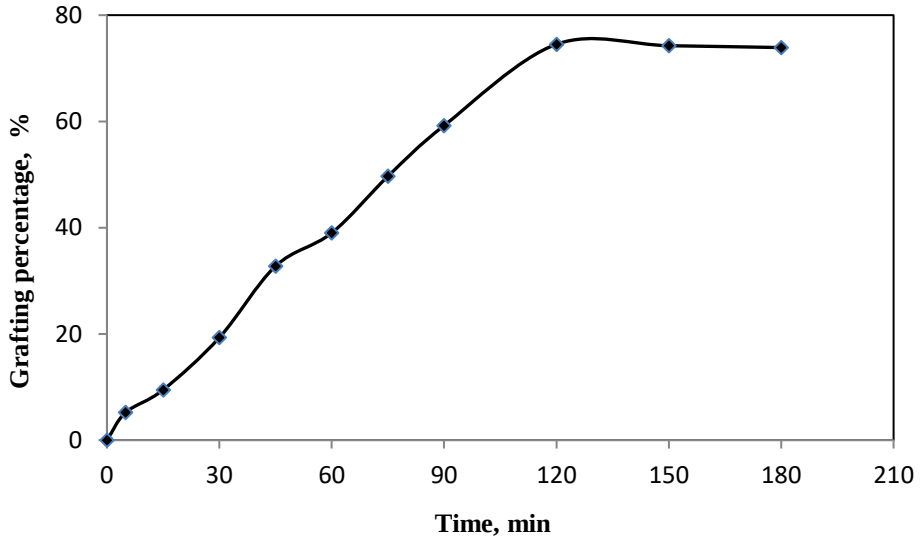


Figure 4. The Effect of Time on Grafting Percentage: [GMA/AAm] = 0.3 M; [BPO] = 1.2×10^{-2}; T = 80 °C

3.5. The Effect of Initiator Concentration on Grafting Percentage

The effect of BPO concentration on the grafting percentage of the GMA/AAm monomer mixture onto PET fibers was investigated, and the results are shown in Figure 5. The maximum grafting percentage (74.13%) was achieved at an initiator concentration of 1.2×10^{-2} M. The grafting percentage increased up to this concentration but decreased at higher initiator concentrations.

The increase in BPO concentration in the polymerization medium led to an increase in the number of free radicals ($C_6H_5COO\bullet$ and $C_6H_5\bullet$). The rise in the number of free radicals enhanced both homopolymeric and copolymeric radical chains, accelerating chain transfer reactions, which in turn increased the grafting percentage. However, at BPO concentrations above the optimal level, the increased radical concentration promoted termination reactions. This likely inhibited the formation of active centers and chain growth on the PET fibers, resulting in a decrease in the grafting percentage. The results are consistent with the literature (Azizinezhad, 2011).

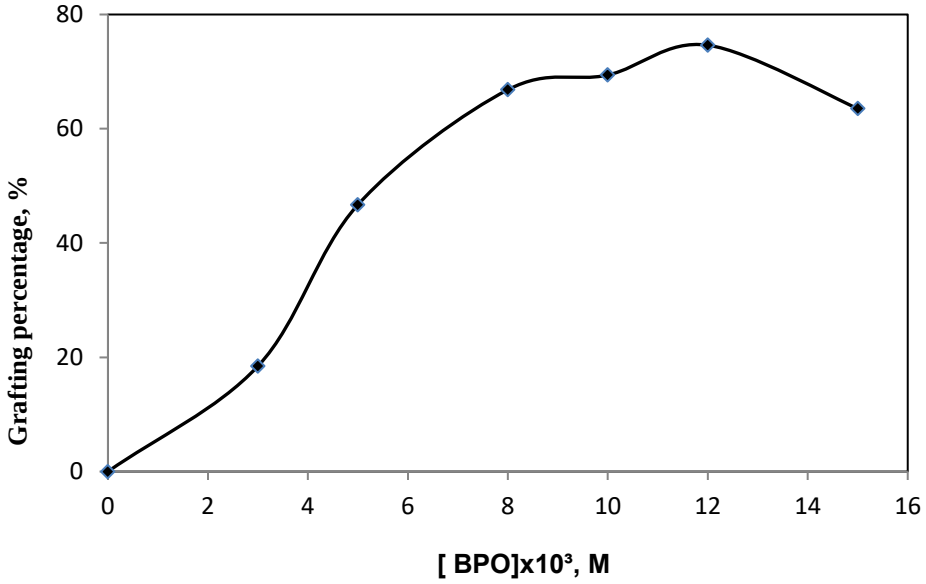


Figure 5. The Effect of Initiator Concentration on Grafting Percentage
[GMA/AAm] = 0.3 M; t = 120 min; T = 80 °C

3.6. The Effect of Monomer Mixture Concentration on Grafting Percentage

The effect of GMA/AAm monomer mixture concentration on grafting percentage was investigated in the concentration range of 0.05–1 M, and the results are presented in Figure 6. It was not possible to work at concentrations above 1 M, as the PET fibers lost their flexibility.

The grafting percentage increased with the rise in the GMA/AAm monomer mixture concentration, reaching up to 178.27% at a 1 M concentration. It is thought that with the increase in monomer concentration, the number of monomer molecules diffusing into the PET fibers also increased, leading to an increase in the number of active centers on the PET fibers. The formation of new active centers on the PET fibers likely facilitated the grafting of monomer molecules onto the fibers, thereby contributing to the increase in the grafting percentage (Seko vd., 2010).

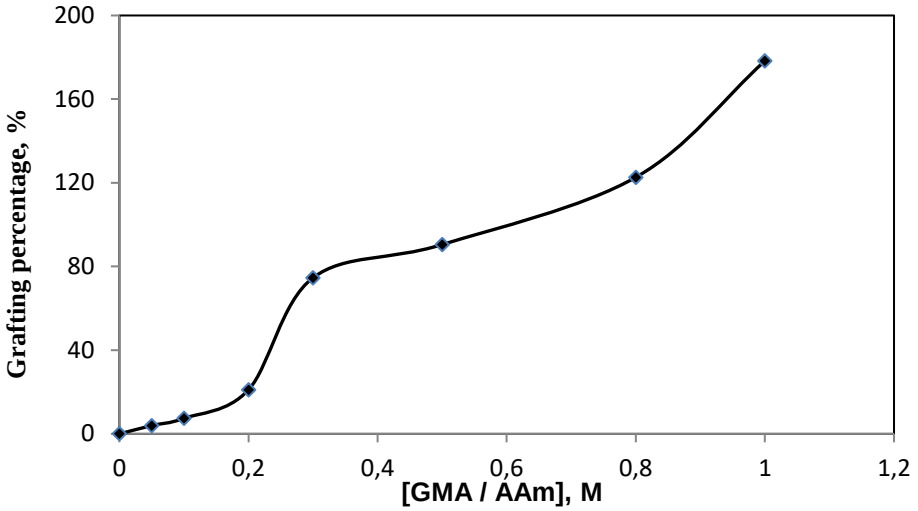


Figure 6. The Effect of Monomer Mixture Concentration on Grafting Percentage
[GMA/AAm] = 30/70 mol; [BPO] = 1.2×10^{-2} M; t = 120 min; T = 80 °C

3.7. Determination of Water Absorption Capacities of Grafted PET Fibers

As a result of grafting the GMA/AAm monomer mixture onto PET fibers, an improvement in the hydrophilic properties of the PET fibers was observed. The relationship between the grafting percentages and water absorption percentages of PET fibers grafted with the GMA/AAm (30/70 mol) monomer mixture is shown in Figure 7.

As seen in Figure 7, as the grafting percentage increases, the water absorption percentage of GMA/AAm-grafted PET fibers also increases. While the water absorption percentage of ungrafted PET fibers is 15.15%, it reaches 61.33% for PET fibers with a grafting percentage of 122.64%.

It is believed that the increase in water absorption percentage is due to the incorporation of polar $–NH_2$ groups into the fiber structure as a result of grafting the GMA/AAm monomer mixture onto the PET fibers (Xu vd., 2016; Aziznezhad, 2014).

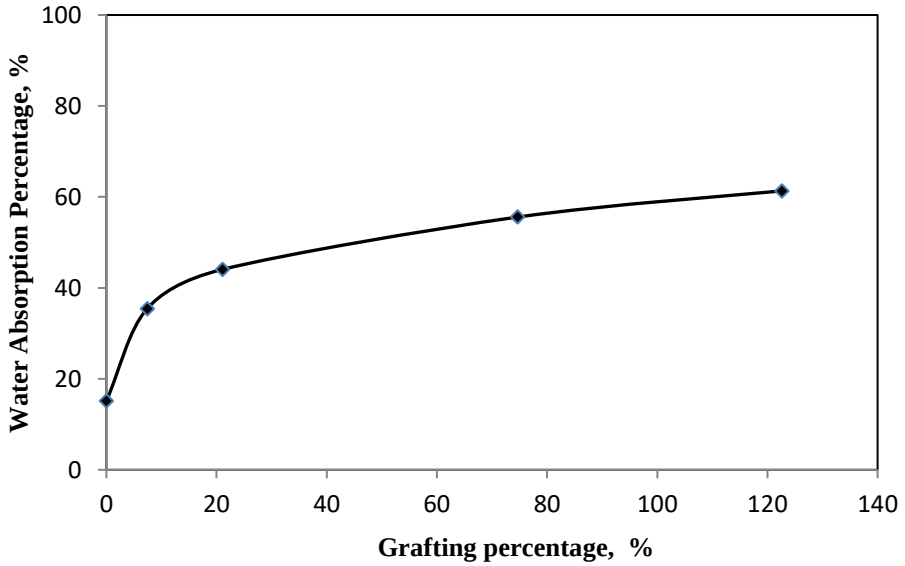


Figure 7. Variation of Water Absorption Percentage with Grafting Percentage

3.8. Dyeability of Fibers

PET fibers, which can only be dyed with disperse dyes due to the lack of functional groups that can interact with dye molecules, were grafted with a GMA/AAm monomer mixture. This process introduced polar epoxy and $-NH_2$ groups onto the fibers. As a result of these groups formed on the PET fibers, they were made dyeable with both basic and acidic dyes.

Figure 8 illustrates the dyeability of PET fibers grafted with a GMA/AAm monomer mixture at specific grafting percentages using acidic and basic dyes. It was observed that the grafting of PET fibers with the GMA/AAm monomer mixture enabled the fibers to be effectively dyed with both the acidic dye Congo red and the basic dye methylene blue. Additionally, the dyeing capacity of GMA/AAm-grafted PET fibers increased with the grafting percentage.

The dyeability of PET fibers grafted with GMA/AAm at a grafting percentage of 122.64% was determined to be 11.99 mg dye/g PET fiber for Congo red and 9.12 mg dye/g PET fiber for methylene blue. The grafting of PET fibers with the GMA/AAm monomer mixture introduced epoxy and -NH_2 groups on the fibers, which can interact more effectively with dye molecules. It is thought that the presence of these polar groups enhances the dyeability of PET fibers after grafting (Kushwaha vd., 2010; Prachayawarakorn vd., 2006; Bozkaya, vd., 2021; Arslan, vd., 2019).

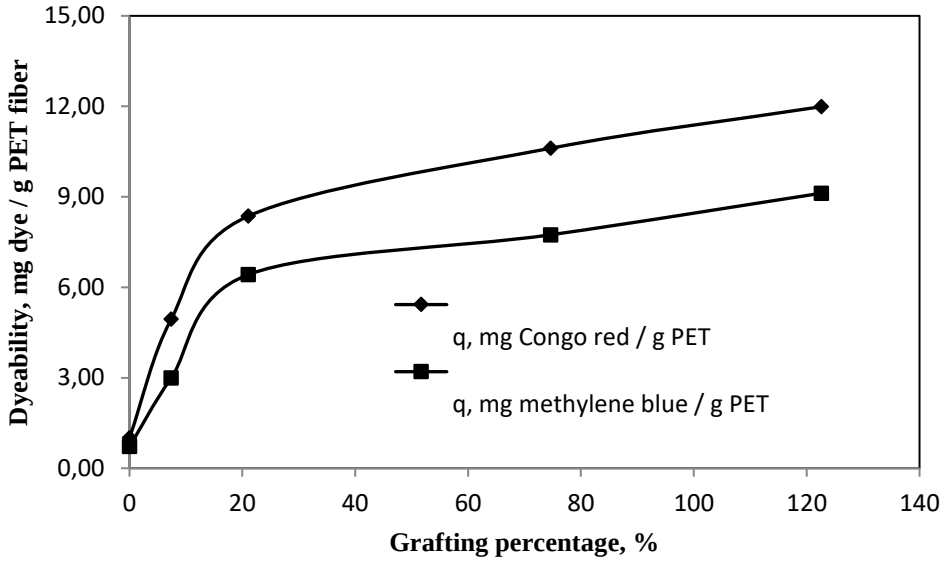
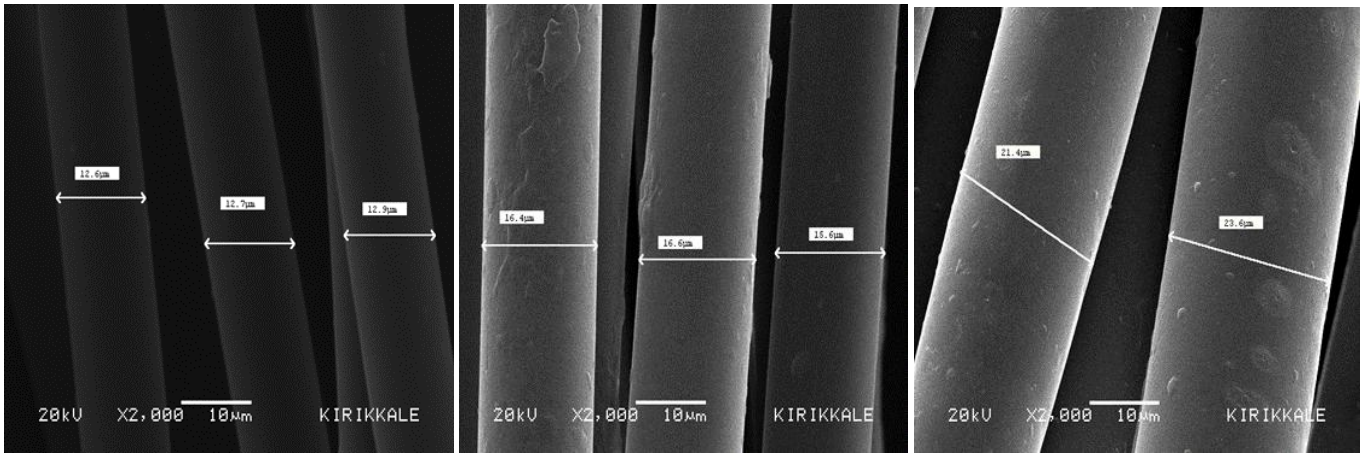


Figure 8. Variation of the dyeability of GMA/AAm-grafted PET fibers with grafting percentage
Dyeing conditions for the fibers: Congo red: pH = 5, V = 25 mL, time = 60 min, t = 25 °C
Methylene blue: pH = 10, V = 25 mL, time = 60 min, t = 25 °C

3.9. Measurement of Fiber Diameters and SEM Images

The diameter values of PET fibers grafted with a GMA/AAm monomer mixture at different grafting percentages are provided in Table 2. It can be seen from Table 2 that the fiber diameter values increase with the grafting percentage. While ungrafted fibers had a diameter of 12.7 μm, the diameter of GMA/AAm-grafted PET fibers with a grafting percentage of 64.08% increased to 22.5 μm. The results are consistent with the literature (Arslan ve Günay, 2017).

SEM images of ungrafted and GMA/AAm monomer mixture-grafted PET fibers at specific grafting percentages are presented in Figure 9. As seen in the SEM images, the surface of ungrafted PET fibers (Figure 9A) appears smooth, whereas the surfaces of PET fibers with different grafting percentages (Figures 9B and 9C) are relatively rougher. The SEM images of the fiber samples serve as additional evidence of the grafting of the GMA/AAm monomer mixture onto PET fibers.



A **B** **C**

Figure 9. A) Ungrafted PET, B) PET grafted with GMA/AAm at 45.71%, C) PET grafted with GMA/AAm at 64.08%

3.10. Determination of Tg Values of Grafted PET Fibers

The diameter and Tg values of ungrafted and GMA/AAm-grafted PET fibers are provided in Table 2. Additionally, the DSC thermograms of the same samples at specific grafting percentages are shown in Figure 10. Table 2 indicates that as the grafting

percentage increases, both the fiber diameters and Tg values increase. The incorporation of α-methyl, carbonyl groups, and highly polar side groups into the structure of PET fibers has increased the Tg values of the grafted fibers (Coşkun ve Akdeniz, 2010).

Table 2. Diameter and Tg values of GMA/AAm-grafted PET fibers

Grafting percentage (%)	diameter (μm)	T_g (°C)
Ungrafted	12,7	78,36
15,18	13,5	97,25
29,74	14,3	98,30
45,71	16,2	119,80
64,08	22,5	120,51

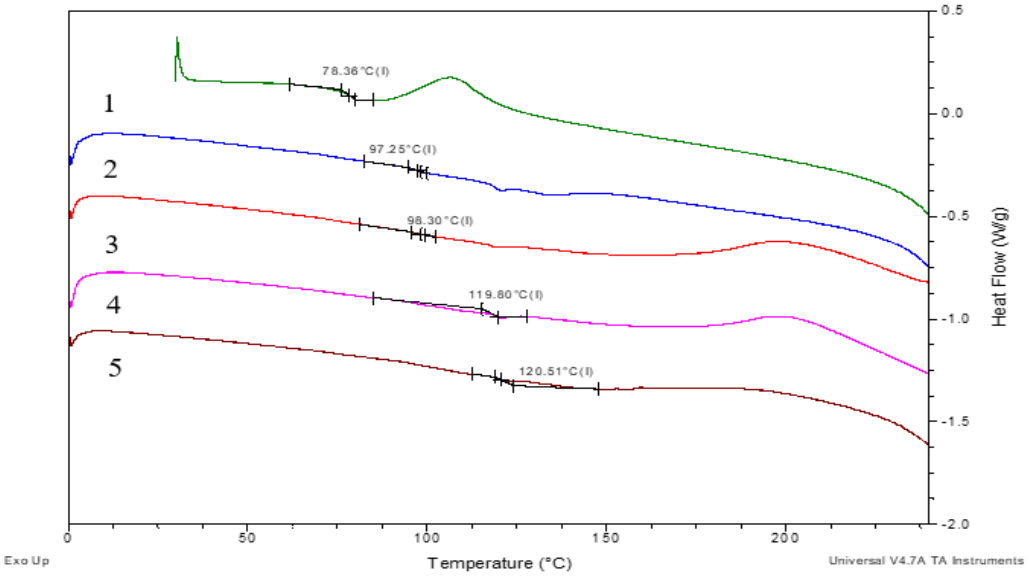


Figure 10. DSC Thermograms of ungrafted and GMA/AAm-g-PET fibers with different grafting percentages: Pure PET (1), 15.18% (2), 29.74% (3), 45.71% (4), 64.08% (5)

3.11.Thermogravimetric Analysis Results

Figure 11 shows the TGA thermograms of ungrafted PET fibers and PET fibers grafted with a GMA/AAm mixture at specific grafting percentages. Table 3 was created based on the data obtained from Figure 11.

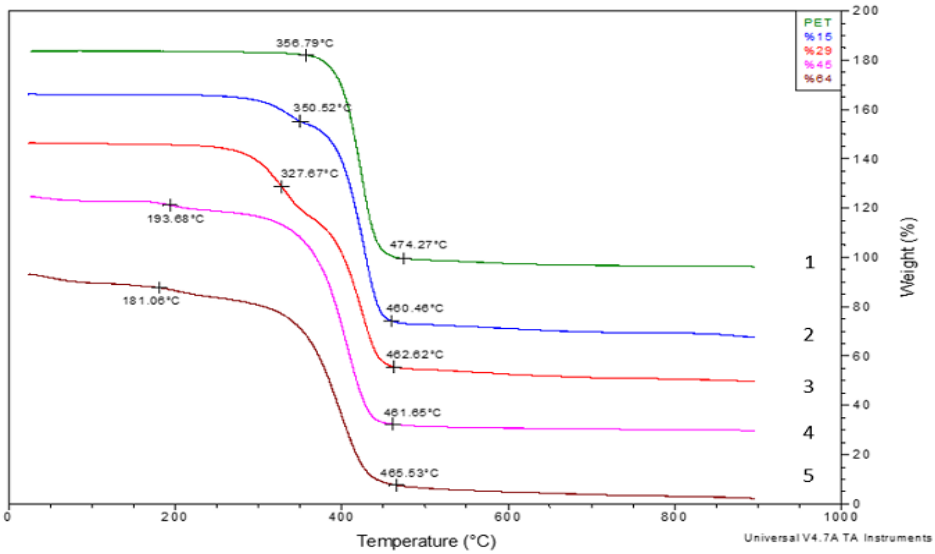


Figure 11. TGA thermograms of ungrafted PET fibers and GMA/AAm-grafted PET fibers with specific grafting percentages: ungrafted PET (1), 15.18% (2), 29.74% (3), 45.71% (4), 64.08% (5).

As seen in Table 3, the decomposition onset temperatures of GMA/AAm-grafted PET fibers with different grafting percentages decrease as the grafting percentage increases. However, the residual amount after decomposition increases with grafting. These results indicate that the thermal stability of ungrafted PET fibers is higher compared to GMA/AAm-grafted PET fibers. The obtained results are observed to be consistent with the literatüre (Coşkun, 2008; Alakara vd., 2008).

Table 3. Thermogram data of GMA/AAm-grafted PET fibers with different grafting percentages

Grafting percentage, %	Initial Decomposition Temperature (IDT, ºC)	Finish Decomposition Temperature (FDT ºC)	Mass Loss, %	Residual, %
0	356	474	87,33	12,67
15,18	350	460	86,08	13,92
29,74	327	462	85,48	14,52
45,71	193	461	84,34	15,66

3.12.Preparation of Enzyme Support Material

Due to its higher nitrogen content based on elemental analysis, GMA/AAm-grafted PET fibers with a (70/30 mol) ratio were used as the enzyme support material in this study. A covalent bonding method was employed to bind the enzyme to the support material. Enzyme immobilization was performed by modifying GMA/AAm-grafted PET fibers as described in Methods 1, 2, and 3. Among these methods, the highest enzyme activity was achieved using Method 3. The activities exhibited by GMA/AAm-grafted PET fibers after modification using different methods are shown in Figure 12.

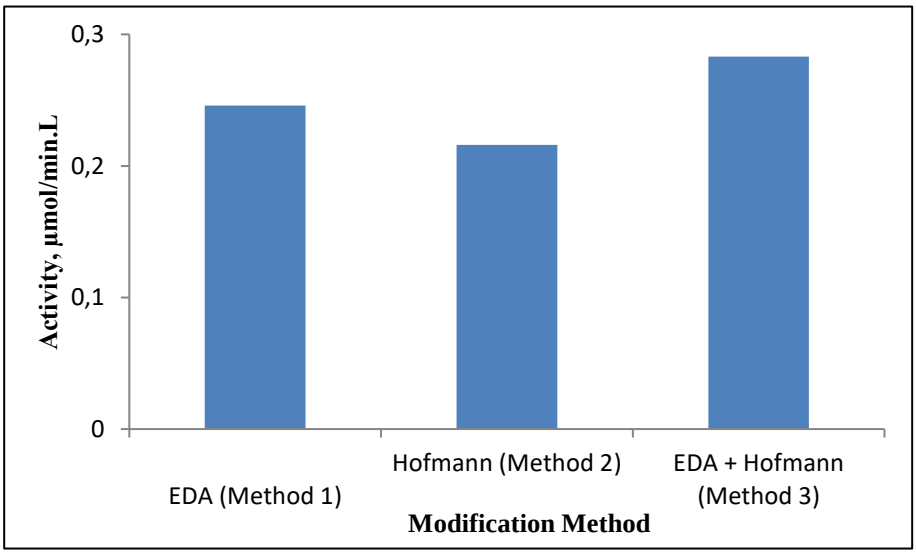


Figure 12. Effect of different modification methods on lipase activity

As seen in Figure 12, the highest enzyme activity was obtained by immobilizing lipase enzyme onto PET fibers modified using both ethylenediamine and the Hofmann rearrangement reaction. It is thought that the combined use of both methods on grafted PET fibers resulted in the formation of more amine groups, allowing more enzymes to immobilize onto the structure via these groups, leading to higher activity. Since the highest activity (Method 3) was achieved by immobilizing the enzyme onto fibers modified with both ethylenediamine and the Hofmann rearrangement reaction, fibers modified in this way were used in the subsequent stages of the study. The proposed reaction steps used in immobilization are shown in Figure 13.

3.13.Enzyme Immobilization and Optimization of Immobilization Conditions

The effect of pH on lipase enzyme immobilization was investigated, and the relationship between relative activity and pH is presented in Figure 14. As shown in Figure 14, enzyme activity varied depending on the pH value of the immobilization environment. The best lipase immobilization onto GMA/AAm-grafted PET fibers was achieved at pH = 5.

The point on the enzyme molecule where the covalent bond forms may depend on the pH value of the immobilization solution. It can be assumed that at these pH values, the active site of the enzyme is masked, preventing covalent bonding through this region. It is hypothesized that the free aldehyde groups on the PET fiber react with amine groups located in regions of the enzyme distant from its active site (Fernandes, 2003).

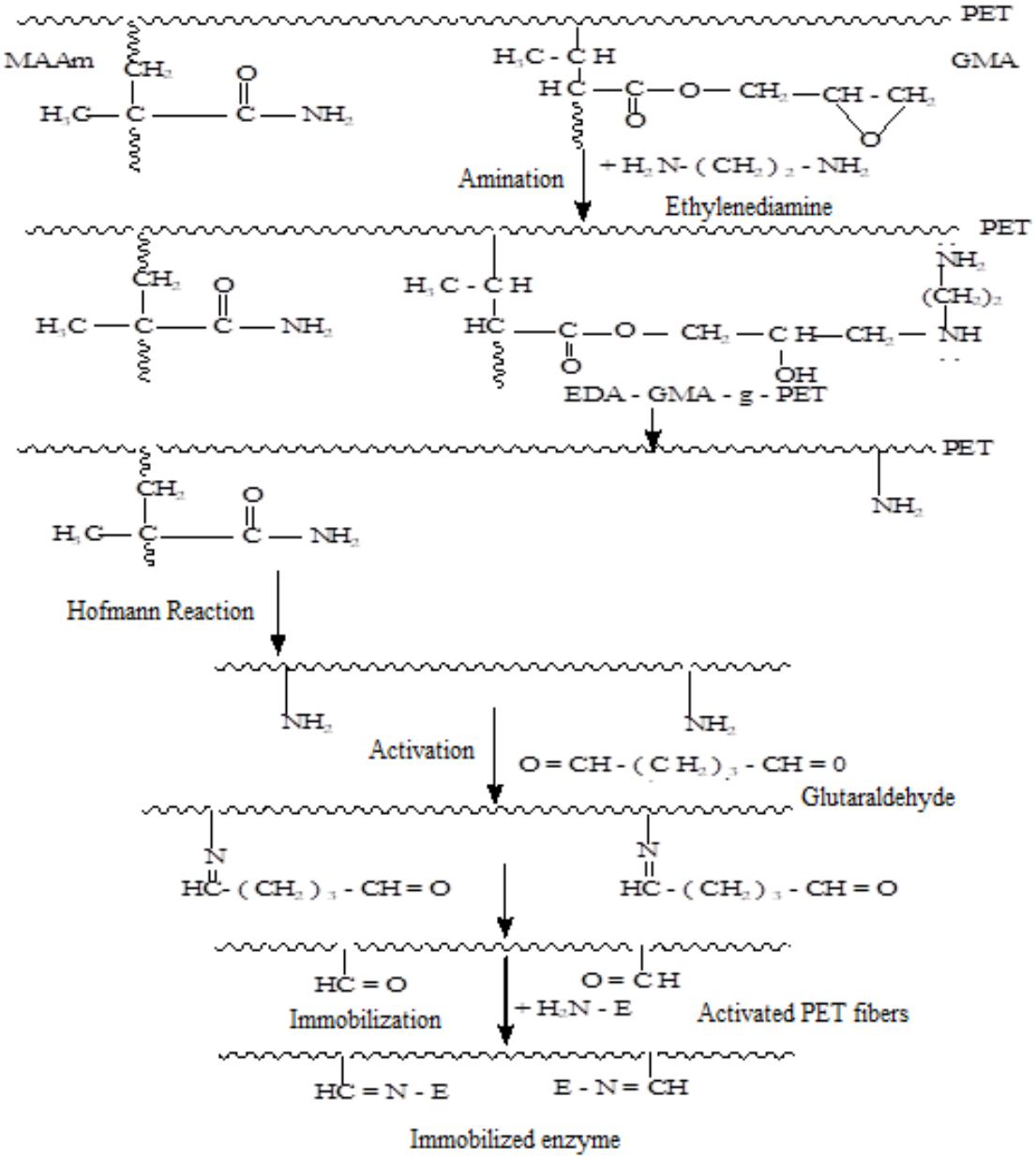


Figure 13. Proposed enzyme immobilization mechanism for modified PET fibers

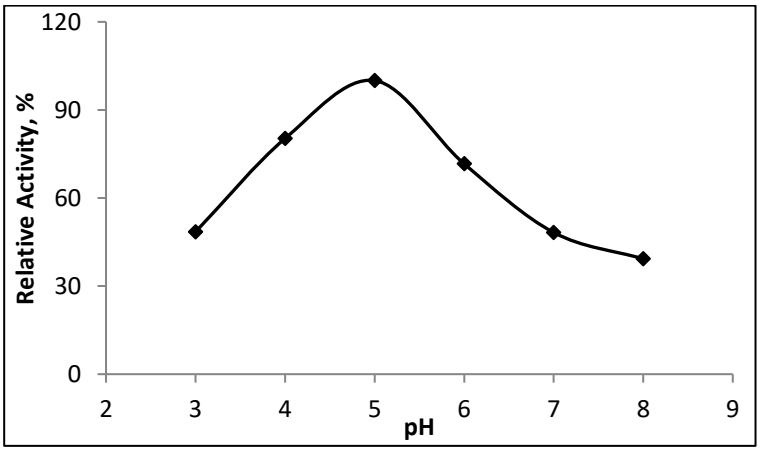


Figure 14. Variation of Relative Activity with pH

The effect of grafting percentage on lipase immobilization onto modified PET fibers was investigated, and the variation of grafting percentage with relative activity is presented in Figure 15. It was observed that as the grafting percentage increases, the activity of the lipase enzyme also increases up to a certain level.

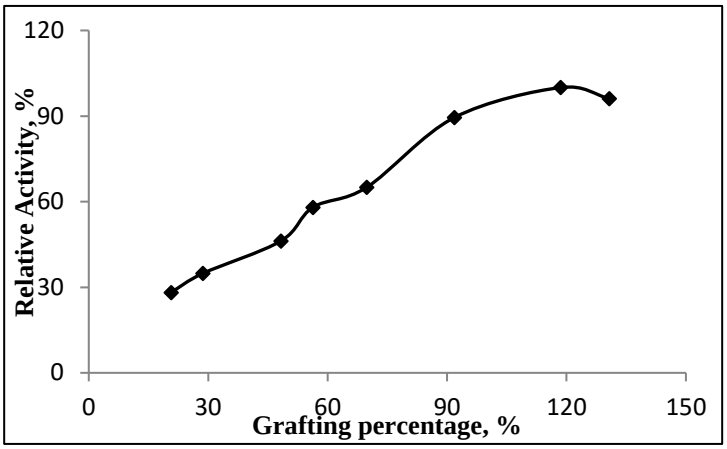


Figure 15. Variation of Grafting Percentage with Relative Activity

The amount of lipase enzyme immobilized onto modified PET fibers is proportional to the number of functional groups on the fibers. The increase in amide and epoxy groups on the surface of modified PET fibers also indicates an increase in amine groups formed as a result of EDA and Hofmann rearrangement reactions on the fiber surface. Therefore, an increase in enzyme activity has been observed depending on the number of functional groups.

3.14. Effect of pH on Enzyme Activity

The variation in the activities of free and immobilized lipase enzymes with respect to solution pH is presented in Figure 16. As shown in Figure 16, the pH values at which free and immobilized lipase enzymes exhibit maximum activity are 6 and 7, respectively. Depending on the support materials used in enzyme immobilization, changes in the optimum pH values of immobilized enzymes compared to free enzymes can occur. This is because enzyme activity is significantly influenced by environmental conditions (Atia, 2003). A change occurred in the H profile of the lipase enzyme immobilized onto GMA/AAm-grafted PET fibers. The observation of maximum activities of free and immobilized lipase enzymes at different pH values is attributed to the differences in the distribution of hydrogen ions between the microenvironment of the support material and the solution in immobilized systems (Cheung, 2024).

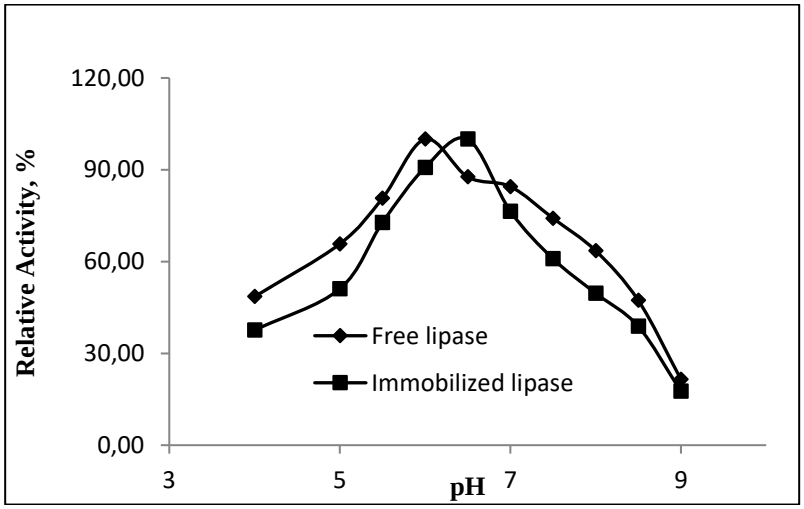


Figure 16. Effect of pH on the Activity of Free and Immobilized Lipase Enzymes

3.15.Effect of Temperature on Enzyme Activity

The effect of temperature on the activity of free and immobilized lipase enzymes was examined in the range of 20-70 °C, and the results are presented in Figure 17. As shown in Figure 17, the maximum activity for both free and immobilized lipase was achieved at a temperature of 50 °C.

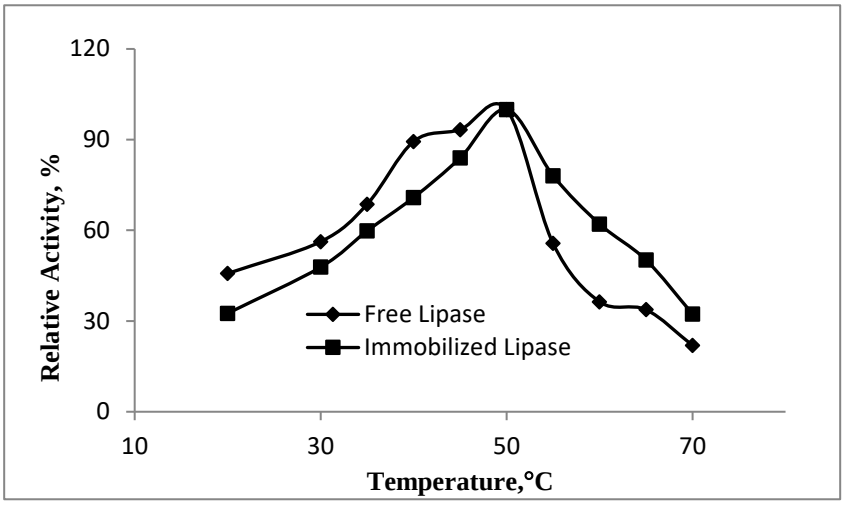


Figure 17. Effect of Temperature on the Activity of Free and Immobilized Lipase Enzymes

The catalytic activities of enzymes are related to their conformational structures. Similar to chemical catalysts, the activities of enzymes increase with rising temperatures. However, enzymes are generally unstable at high temperatures. They tend to become unstable above the temperature at which they exhibit maximum activity, as structural disruptions occur in their conformational arrangements at these temperatures. Immobilization often provides enzyme molecules with a more stable and rigid structure. In this study, the temperature at which both free and immobilized enzymes exhibited maximum activity was found to be 50 °C. The results are consistent with the literature (Fernández, 2021).

3.16.Use of Lipase Enzyme in the Hydrolysis of Different Oil Types

In this study, the effects of free and immobilized lipase enzymes on the hydrolysis of various oil types were investigated. In the hydrolysis experiments, each oil type was used separately as a substrate. The variation in the total amount of fatty acids produced with the type of oil used is presented in Figure 18.

In Figure 18, it can be observed that free and immobilized lipase enzymes produced the highest amount of fatty acids in corn oil and the lowest amount in soybean oil. The amounts of fatty acids produced by the lipase enzymes under specific conditions were found to be 4.42 mg and 2.96 mg for free and immobilized enzymes, respectively. This demonstrates that lipase enzymes immobilized onto the mentioned support materials can be effectively used for the hydrolysis of oils.

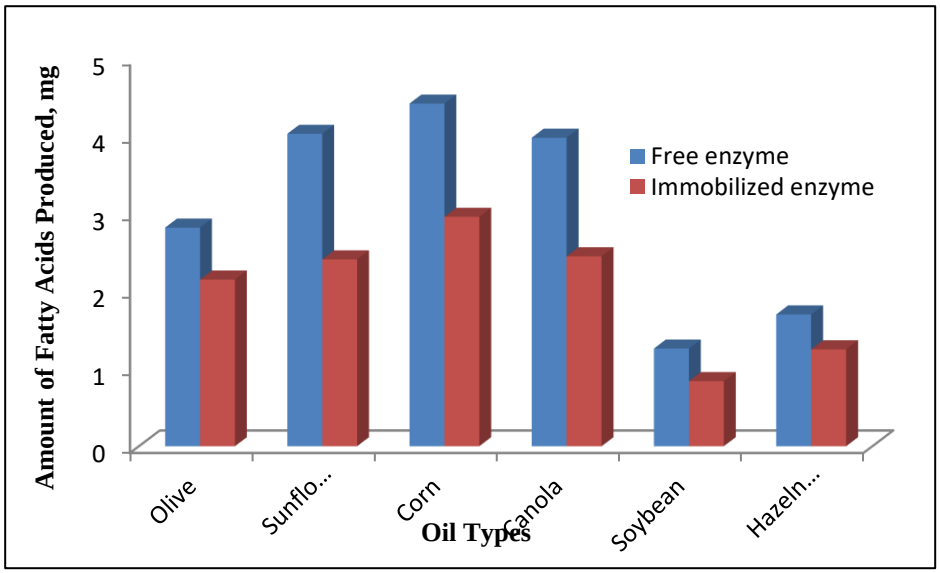


Figure 18. Variation of Fatty Acid Production with Oil Type
[Substrate] = 0.45 g/mL, Temperature = 50 °C, Time = 30 min

In general, most lipases easily perform the hydrolysis of medium-chain oils. While some lipase enzymes cleave short-chain fatty acids, they may exhibit no activity for long-chain fatty acids. Additionally, the source from which the lipase enzyme is derived influences its selectivity toward fatty (Al-Taweel, 1995).

3.17.FTIR Analysis of PET Fibers

Figure 19 presents the FTIR spectra of ungrafted PET fiber (A), GMA/AAm grafted PET fiber (B), PET fiber modified via EDA and Hofmann rearrangement reaction (C), and PET fiber activated with glutaraldehyde (D). In spectrum B, the stretching peak of the carbonyl group from acrylamide (~1664 cm^{-1}) and the unassociated N−H vibration peaks (~3337 and 3208 cm^{-1}) can be observed. These data confirm that acrylamide has been grafted onto the PET fiber. Additionally, the peak observed at ~904 cm^{-1} in spectrum B is a specific peak attributed to the epoxy ring in GMA (Madrid vd., 2013). This peak is evidence that GMA has been grafted onto the PET fiber. When spectra B and C are examined, a decrease in the intensity of the peak corresponding to the carbonyl group (~1664 cm^{-1}) is observed. Furthermore, the N−H vibration peaks at 3337 and 3208 cm^{-1} shift to a broader associated N−H vibration peak at 3275 cm^{-1}. From these peaks, it can be inferred that some of the acrylamide groups on the PET fiber have been converted to amine groups via the EDA and Hofmann reaction. When spectra C and D are compared, the inclusion of glutaraldehyde in the structure results in the appearance of the characteristic peak of glutaraldehyde (C-H stretching vibration of −CHO) at a wavelength of 2869 cm^{-1}. Additionally, an increase in the intensity of the peak at 2936 cm^{-1}, attributed to the C−H stretching vibration of $-CH_2-$, is observed due to the incorporation of glutaraldehyde into the structure.

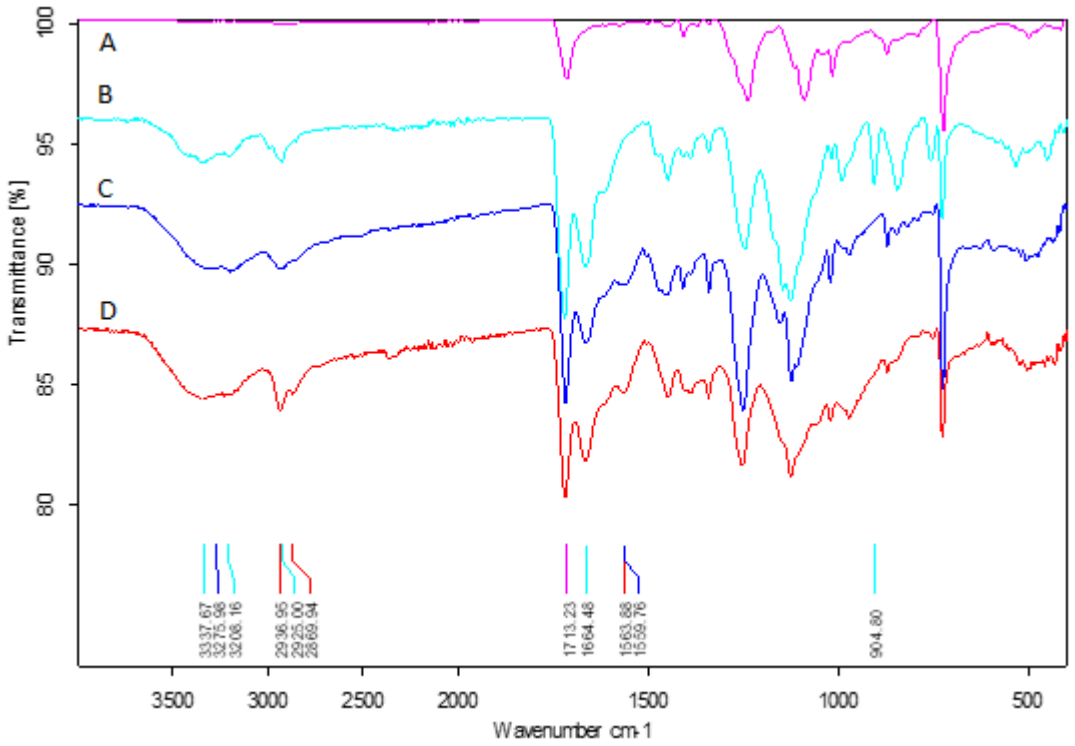


Figure 19. FTIR spectra of ungrafted PET fiber (A), PET-g-GMA/AAm PET fiber (B), PET fiber modified with EDA and Hofmann (C), and PET fiber activated with glutaraldehyde (D).

The findings of this study can be summarized as follows:

1. GMA/AAm monomer mixture was grafted onto PET fibers in an aqueous medium using BPO as the initiator.
2. The highest grafting percentage of the GMA/AAm monomer mixture onto PET fibers was found to be 177.75% with a GMA/AAm (90/10 mol) ratio. The optimal grafting conditions were determined as [BPO] = 1.2×10^{-2} M, [GMA/AAm] = 0.3 M, t = 120 minutes, and T = 80 °C.
3. As the grafting percentage of GMA/AAm monomer mixture onto PET fibers increased, the fiber diameters, water absorption capacities, and Tg values also increased.
4. Due to the synergistic effect, the grafting percentage of AAm onto PET fibers in the presence of GMA was observed to be significantly higher compared to its grafting percentage when used alone.
5. It was observed that grafting improved the dyeability of PET fibers with both acidic and basic dyes.
6. TGA thermograms revealed that ungrafted PET fibers exhibited higher thermal stability compared to GMA/AAm grafted PET fibers.
7. SEM images showed that the diameters of PET fibers increased after grafting with the GMA/AAm monomer mixture and that the fibers exhibited a relatively more heterogeneous surface after grafting.

8. Various modification methods were tested to introduce suitable functional groups onto the monomer mixture-grafted PET fibers, and the highest enzyme activity was obtained when the fibers were first modified with EDA and then through Hofmann rearrangement.
9. The best lipase immobilization on the support material was observed at pH = 5. It was found that the immobilized lipase enzyme activity depended on the grafting percentage of the PET fiber. Enzyme activity increased up to a certain grafting percentage and then stabilized.
10. The pH value at which the free lipase enzyme exhibited maximum activity shifted from 6 to 7 after immobilization.
11. The temperature at which both free and immobilized lipase enzymes exhibited maximum activity was found to be 50 °C.
12. It was observed that the immobilized lipase enzyme hydrolyzed various types of oils in the study.

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