ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL

THE EFFECT OF PREPARATION METHODS ON THE MORPHOLOGICAL AND RHEOLOGICAL PROPERTIES OF PHBH/CNC NANOCOMPOSITES

M.Sc. THESIS

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★LİSANSÜSTÜ EĞİTİMENSTİTÜSÜ

HAZIRLAMA YÖNTEMLERİNİN PHBH/CNC NANOKOMPOZİTLERİNİN MORFOLOJİK VE REOLOJİK ÖZELLİKLERİ ÜZERİNDEKİ ETKİSİ

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ŞUBAT 2026

Onur KÜÇÜKSUBAŞI, a M.Sc. of İTU Graduate School student ID 506231416, successfully defended the thesis/dissertation entitled "THE EFFECT OF PREPARATION METHODS ON THE MORPHOLOGICAL AND RHEOLOGICAL PROPERTIES OF PHBH/CNC NANOCOMPOSITES", which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Date of Submission : 03 February 2026
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To my family,

FOREWORD

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I dedicate this thesis to my beloved family, whose unconditional love and constant support have been my greatest source of strength throughout my life. My mother Emine KÜÇÜKSUBAŞI, My father Nail KÜÇÜKSUBAŞI and My brother Oğuz KÜÇÜKSUBAŞI.

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ABBREVIATIONS

3HB **:** 3-Hydroxybutyrate
3HH **:** 3-Hydroxyhexanoate
BNC **:** Bacterial Nanocellulose
CHL **:** Chloroform
CNC **:** Cellulose Nanocrystals
CNF **:** Cellulose Nanofibers
CNT **:** Carbon Nanotubes
DMF **:** Dimethylformamide
DMSO **:** Dimethyl Sulfoxide
DSC **:** Differential Scanning Calorimetry
FTIR **:** Fourier Transform Infrared Spectroscopy
GO **:** Graphene Oxide
MCL **:** Medium-Chain-Length
MFI **:** Melt Flow Index
MFR **:** Melt Flow Rate
MMT **:** Montmorillonite
MW **:** Molecular Weight
NC **:** Nanocomposite
OMMT **:** Organically Modified Montmorillonite
PHA **:** Polyhydroxyalkanoate
PHB **:** Poly(3-hydroxybutyrate)
PHBH **:** Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)
PHBV **:** Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
ppm **:** Parts per million
Tc : Crystallization Temperature
Tg **:** Glass Transition Temperature
TG/TGA **:** Thermogravimetric Analysis
THF **:** Tetrahydrofuran
Tm **:** Melting Temperature

SYMBOLS

η*	**:** Complex viscosity
G′	**:** Storage modulus
G″	**:** Loss modulus
G*	**:** Complex modulus
ω	**:** Angular frequency
t½	**:** Crystallization half-time
wt.%	**:** Weight fraction
mol%	**:** Mole fraction
mcG	**:** Percolation threshold
βcG	**:** Percolation constant
n	**:** Power-law exponent
σy	**:** Apparent yield stress
T	**:** Temperature
ΔHc	**:** Crystallization enthalpy
ΔHm	**:** Melting enthalpy
Xc	**:** Degree of crystallinity
ρ	**:** Density
ε	**:** Dielectric constant
E	**:** Young's modulus
σ	**:** Stress
εb	**:** Elongation at break

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THE EFFECT OF PREPARATION METHODS ON THE MORPHOLOGICAL AND RHEOLOGICAL PROPERTIES OF PHBH/CNC NANOCOMPOSITES

SUMMARY

The growing use of conventional plastics has become a serious environmental problem. Most of these materials are produced from petroleum and remain in the environment for many years after use. As a result, plastic waste accumulates in landfills, soil, and oceans, causing long-term pollution and ecological damage. At the same time, the dependence on fossil resources increases carbon emissions and limits sustainability. For these reasons, there is a strong need to develop alternative materials that are both environmentally friendly and derived from renewable sources.

Biodegradable polymers offer an important solution to this problem. Polyhydroxyalkanoates (PHAs) have attracted great interest because they are produced by microorganisms and can fully degrade under natural conditions. Poly(3-hydroxybutyrate) (PHB) is a key member of the PHA family and has attracted strong industrial interest because it is biodegradable and hydrophobic. To overcome some of its limitations, a copolymer known as poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH) was developed. The presence of 3-hydroxyhexanoate (3HH) units in the polymer chain increases flexibility and toughness compared with PHB. These units also reduce brittleness and improve processability, which makes PHBH more suitable for applications such as packaging, agricultural films, and medical products.

Despite these advantages, PHBH still has some limitations. Its mechanical strength is lower than that of many conventional plastics, and it shows slow crystallization and weak melt stability. These properties make processing difficult and limit its use in more demanding applications. Therefore, improving the performance of PHBH while keeping its biodegradable nature is an important research goal.

One effective way to enhance polymer properties is the preparation of nanocomposites. In this approach, small amounts of nanoparticles are added to the polymer matrix. Even at low concentrations, these fillers can significantly improve stiffness, strength and thermal stability. Among the available nanofillers, cellulose nanocrystals (CNCs) are especially attractive. CNCs are obtained from natural cellulose, which is abundant, renewable, and biodegradable. They are lightweight, stiff, and have a large surface area, which allows strong interactions with polymer chains.

When CNCs are well dispersed in a polymer, they can greatly improve its mechanical and thermal properties. They can also influence the crystallization behavior of the polymer by acting as nucleation agents. However, CNCs tend to form agglomeration because of strong hydrogen bonding between their surfaces. This makes it difficult to achieve a uniform dispersion, especially in hydrophobic polymers such as PHBH. Therefore, the processing method and the choice of solvent play a crucial role in determining the final properties of the nanocomposites.

In this study, PHBH/CNC nanocomposites were prepared using the solution casting method. This technique allows better control over CNC dispersion compared to melt processing, particularly for hydrophilic fillers. Four different solvents were used: tetrahydrofuran (THF), chloroform (CHL), dimethylformamide (DMF), and dimethyl sulfoxide (DMSO). These solvents have different polarities and solvating abilities, which can strongly affect how PHBH and CNCs interact with each other.

First, the influence of solvent type on CNC dispersion and melt behavior was examined. For this purpose, nanocomposites containing 3 wt.% CNC were prepared using each solvent. Rheological measurements were then carried out to study the internal structure of the materials. Rheological behavior is very sensitive to filler dispersion. When CNCs are poorly dispersed, the material behaves similarly to neat PHBH. When CNCs are well distributed and interact with each other, the melt becomes more elastic and more resistant to flow.

The results showed that the solvent had a strong effect on composite structure. Samples prepared with THF and CHL showed only small changes in rheological behavior, indicating limited CNC dispersion. DMF performed better, leading to higher viscosity and elasticity. The best results were obtained with DMSO. In this case, the nanocomposites showed a strong increase in elastic behavior at low frequencies, which suggests the formation of a well-connected CNC network inside the PHBH matrix.

The superior performance of DMSO can be explained by its strong ability to interact with both PHBH and CNCs. DMSO can effectively weaken the hydrogen bonds between CNC particles, reducing their tendency to agglomeration. After identifying DMSO as the most suitable solvent, nanocomposites with different CNC contents (0.1, 1, 3, and 5 wt.%) were prepared. Rheological analysis showed that increasing the CNC content gradually changed the melt behavior from liquid-like to more solid-like. At a very low CNC concentration, a continuous filler network was formed, known as the percolation threshold. This value was found to be extremely low, showing that CNCs are very effective when they are well dispersed.

Thermal analysis also revealed that CNCs improved the crystallization behavior of PHBH. The crystallization temperature increased and the crystallization process became faster, which is beneficial for industrial processing. These effects are related to the nucleating ability of CNCs, which help polymer chains organize more easily into crystalline regions.

Overall, this study shows that the properties of PHBH can be greatly enhanced by adding small amounts of cellulose nanocrystals. The effectiveness of this improvement strongly depends on the solvent used during processing. Among the solvents examined, DMSO led to the best CNC dispersion and the most noticeable gains in rheological and thermal performance. These results demonstrate that PHBH/CNC nanocomposites have strong potential as sustainable, high-performance materials. By carefully adjusting the solvent and CNC content, the properties of PHBH can be tailored for more demanding uses while still preserving its biodegradable and environmentally friendly nature. This strategy supports the development of advanced bioplastics that can reduce dependence on conventional petroleum-based polymers and promote more sustainable material.

HAZIRLAMA YÖNTEMLERİNİN PHBH/CNC NANOKOMPOZİTLERİNİN MORFOLOJİK VE REOLOJİK ÖZELLİKLERİ ÜZERİNDEKİ ETKİSİ

ÖZET

Geleneksel plastiklerin giderek artan kullanımı, günümüzde önemli bir çevresel sorun haline gelmiştir. Bu malzemelerin büyük bir kısmı petrol kaynaklıdır ve kullanım sonrası doğada uzun süre bozunmadan kalmaktadır. Bu durum, plastik atıkların düzenli depolama alanlarında, toprakta ve özellikle okyanuslarda birikmesine neden olmakta; uzun vadeli çevresel kirliliğe ve ekolojik zarara yol açmaktadır. Ayrıca fosil kaynaklara bağımlılık, karbon emisyonlarını artırmakta ve sürdürülebilir üretim yaklaşımlarını sınırlamaktadır. Bu nedenle hem çevre dostu hem de yenilenebilir kaynaklardan elde edilen alternatif malzemelerin geliştirilmesi büyük önem taşımaktadır.

Biyobozunur polimerler, bu çevresel problemlere karşı etkili çözümler sunmaktadır. Özellikle polihidroksialkanoatlar (PHA'lar), mikroorganizmalar tarafından doğal yollarla sentezlenebilmeleri ve uygun çevresel koşullar altında tamamen bozunabilmeleri nedeniyle büyük ilgi görmektedir. PHA ailesinin önemli bir üyesi olan poly(3-hydroxybutyrate) (PHB), biyobozunur ve hidrofobik yapısı sayesinde çeşitli endüstriyel uygulamalarda değerlendirilmiştir. Ancak PHB'nin yüksek kırılganlık göstermesi, dar işleme penceresi ve sınırlı mekanik dayanımı, kullanım alanlarını önemli ölçüde kısıtlamaktadır. Bu sınırlamaların aşılması amacıyla, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH) kopolimeri geliştirilmiştir. Zincire 3-hidroksihekzanoat (3HH) birimlerinin dahil edilmesi, polimer zincir hareketliliğini artırmakta; böylece esneklik ve tokluk iyileşmekte, kırılganlık azalmaktadır. Ayrıca işlenebilirlik ve darbe dayanımı da belirgin şekilde geliştirilmektedir. Bu özellikleri sayesinde PHBH; ambalaj malzemeleri, tarımsal filmler, tek kullanımlık ürünler ve biyomedikal uygulamalar gibi birçok alanda potansiyel bir alternatif olarak değerlendirilmektedir.

Buna rağmen PHBH'nin performans açısından bazı sınırlamaları devam etmektedir. Özellikle mekanik dayanım değerleri geleneksel petrol bazlı polimerlere kıyasla daha düşüktür. Ayrıca kristallenme kinetiği nispeten yavaştır ve bu durum üretim sürecinde verimlilik açısından dezavantaj oluşturabilir. Bu nedenle, biyobozunur yapıyı koruyarak malzemenin mekanik, reolojik ve termal performansını artırmak önemli bir araştırma hedefidir.

Polimer özelliklerini geliştirmek için en etkili yöntemlerden biri nanokompozit yaklaşımıdır. Bu yaklaşımda polimer matrise düşük oranlarda nano boyutlu katkı maddeleri eklenir. Nano ölçekli takviyeler, yüksek yüzey alanları sayesinde polimer zincirleriyle güçlü etkileşimler kurarak malzemenin rijitliğini, mukavemetini ve termal kararlılığını artırabilir. Ayrıca reolojik davranış üzerinde de belirgin etkiler göstererek işleme özelliklerini iyileştirebilirler. Bu bağlamda selüloz nanokristalleri (CNC), yenilenebilir kaynaklardan elde edilmeleri, biyobozunur olmaları ve yüksek mekanik dayanımları nedeniyle özellikle dikkat çekmektedir. CNC'ler yüksek özgül yüzey

alanına sahip olup, polimer zincirleri ile hidrojen bağları üzerinden güçlü etkileşimler oluşturabilirler.

CNC'lerin polimer matriste homojen şekilde dağılması durumunda, mekanik dayanımda artış, elastik modülde yükselme ve kristallenme davranışında iyileşme gözlenebilir. Bununla birlikte CNC'ler yüzeylerindeki yoğun hidrojen bağları nedeniyle güçlü aglomerasyon eğilimi göstermektedir. Bu durum özellikle hidrofobik karaktere sahip PHBH gibi polimerlerde dispersiyon problemlerine yol açmaktadır. Dolayısıyla üretim yöntemi ve kullanılan çözücünün seçimi, nanokompozitlerin mikro yapısını ve nihai özelliklerini belirleyen kritik parametrelerdir.

Bu çalışmada PHBH/CNC nanokompozitleri çözelti döküm yöntemi ile hazırlanmıştır. Çözelti bazlı yöntem, özellikle hidrofilik takviyelerin dağılımını kontrol etme açısından eriyik karıştırma tekniklerine kıyasla daha avantajlıdır. Çalışmada farklı polarite ve çözme kabiliyetlerine sahip tetrahidrofuran (THF), kloroform (CHL), dimetilformamid (DMF) ve dimetil sülfoksit (DMSO) çözücüleri kullanılmıştır. Bu çözücülerin fizikokimyasal özellikleri, CNC dispersiyonu ve polimer–takviye malzeme etkileşimleri üzerinde belirleyici rol oynamaktadır.

İlk aşamada çözücü türünün CNC dağılımı ve reolojik davranış üzerindeki etkisi incelenmiştir. Bu amaçla her çözücü ile %3 (ağırlıkça) CNC içeren nanokompozitler hazırlanmıştır. Reolojik analizler, partikül ağ yapısına ve parikül–matris etkileşimlerine son derece duyarlıdır. CNC'lerin iyi dağıldığı sistemlerde düşük frekanslarda elastik davranış artışı ve viskoz akışa karşı direnç gözlenmiştir. Sonuçlar, çözücü türünün kompozit yapısı üzerinde güçlü bir etkisi olduğunu ortaya koymuştur. THF ve CHL ile hazırlanan numunelerde sınırlı iyileşme gözlenirken, DMF ile daha belirgin gelişmeler elde edilmiştir. En yüksek performans ise DMSO kullanılan sistemlerde tespit edilmiştir. Bu sistemlerde düşük frekans bölgesinde belirgin elastik davranış artışı, PHBH matrisi içinde sürekli ve bağlantılı bir CNC ağ yapısının oluştuğunu göstermektedir.

DMSO'nun üstün performansı, yüksek dielektrik katsayısı ve güçlü polar karakterine bağlanabilir. Bu özellikler, CNC yüzeylerindeki hidrojen bağlarını zayıflatarak parçacıklar arası etkileşimi azaltmakta ve aglomerasyonu engellemektedir. Böylece daha homojen bir dispersiyon sağlanmakta ve polimer matrisi içinde perkolasyon ağı oluşumu desteklenmektedir. En uygun çözücü olarak DMSO belirlendikten sonra farklı CNC içeriklerine (%0,1, %1, %3 ve %5) sahip nanokompozitler hazırlanmıştır. Reolojik sonuçlar, CNC miktarı arttıkça malzemenin daha katı benzeri bir davranış sergilediğini ve düşük CNC oranlarında bile perkolasyon ağının oluştuğunu göstermiştir.

Isıl analizler, CNC ilavesinin PHBH'nin kristallenme sıcaklığını artırdığını ve kristallenme hızını iyileştirdiğini ortaya koymuştur. Bu durum, CNC'lerin etkin çekirdekleyici ajan olarak görev yaptığını doğrulamaktadır. Kristallenmenin hızlanması, üretim süreçlerinde çevrim süresini kısaltarak endüstriyel açıdan önemli avantajlar sağlamaktadır. Ayrıca daha düzenli kristal yapı oluşumu, malzemenin mekanik performansını da olumlu yönde etkileyebilir.

Sonuç olarak bu çalışma, düşük miktarda selüloz nanokristali ilavesinin PHBH'nin reolojik ve termal özelliklerini belirgin şekilde geliştirebildiğini göstermektedir. Bu iyileşmenin başarısında çözücü seçimi kritik rol oynamaktadır ve DMSO, CNC dispersiyonu ve ağ oluşumu açısından en etkili ortam olarak öne çıkmıştır. Elde edilen bulgular, PHBH/CNC nanokompozitlerinin sürdürülebilir, çevre dostu ve yüksek performanslı malzeme sistemleri olarak güçlü bir potansiyele sahip olduğunu

göstermektedir. Çözücü türü ve takviye oranının optimize edilmesiyle, PHBH'nin özellikleri daha geniş uygulama alanlarına uyarlanabilirken biyobozunur yapısı korunabilir. Bu yaklaşım, geleneksel petrol bazlı polimerlere olan bağımlılığın azaltılmasına ve daha sürdürülebilir malzeme teknolojilerinin geliştirilmesine katkı sağlamaktadır.

1. INTRODUCTION

The widespread use of petroleum-derived polymers has led to growing environmental concerns, creating an urgent need for more sustainable material solutions. In this context, bio-based and biodegradable polymers have emerged as promising alternatives that can reduce dependence on fossil resources and lower environmental impact [1,2]. Recent data from European Bioplastics indicate that the global production capacity of bioplastics is projected to more than double by 2030, increasing from 2.31 million tons in 2025 to approximately 4.69 million tons [3]. Among biodegradable polymers, polyhydroxyalkanoates (PHAs) have received significant attention because they are fully biodegradable, compostable, and can be synthesized from renewable sources. Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH) is a member of this family and consists of rigid 3-hydroxybutyrate (3HB) segments and more flexible 3-hydroxyhexanoate (3HH) units. The relative amount of these two components strongly influences the overall behavior of the polymer. Increasing the 3HH content lowers the crystallinity of PHBH and leads to improved processability, thermal stability, and ductility [4,5]. Despite these advantages, PHBH still exhibits weaker thermal and mechanical performance compared with conventional petroleum-based plastics. In addition, its slow crystallization rate and limited melt strength result in unsatisfactory rheological behavior, which restricts its use in demanding applications such as packaging, textile products, biomedical devices, and automotive components [6].

To overcome the limitations of PHBH, methods such as blending with other polymers and producing composites or nanocomposites have been used. Among those methods, nano-reinforcement approaches aimed at improving the properties of PHBH is widely preferred because of its advantages. The development of PHBH-based composites containing nanofillers is a widely usedi low cost approach to rheological and crystallization behavior, which leads to better processability[4].

A wide range of nanoscale fillers has been investigated for PHBH-based nanocomposites, including cellulose-derived materials such as cellulose nanocrystals

(CNCs) and cellulose nanofibers (CNFs), inorganic nanoparticles like nanosilica, carbon-based nanomaterials (e.g., carbon nanotubes and graphene oxide), as well as layered clays such as montmorillonite and its organically modified forms. Among these options, CNCs are particularly attractive because they are obtained from renewable and biodegradable resources while also providing outstanding mechanical reinforcement. They possess a highly ordered crystalline structure with crystallinity values exceeding 85%, tensile strength in the gigapascal range, and a high elastic modulus. In addition, their large specific surface area, low thermal expansion, and high aspect ratio enable efficient stress transfer and strong interfacial interactions within polymer matrices [7].

PHBH/CNC nanocomposites can be fabricated using either melt mixing or solution casting techniques. CNC particles contain a high density of surface hydroxyl (–OH) groups, which leads to strong hydrogen bonding and gives them a highly hydrophilic character. When CNCs are processed with hydrophobic thermoplastic matrices through melt mixing, these strong interparticle interactions promote aggregation of the nanoparticles. The formation of such agglomerates negatively affects stress transfer within the composite and results in a deterioration of the mechanical properties [8,9].

In comparison, solution casting provides a more favorable environment for CNC dispersion, as the presence of a solvent reduces particle–particle interactions and facilitates a more uniform distribution within the PHBH matrix. Nanoparticle dispersion is also influenced by the molecular weight of the polymer; lower molecular weight systems generally exhibit higher melt flow index (MFI), which reflects increased chain mobility during processing. Therefore, PHBH X331A, with its relatively high MFI, was selected to promote improved dispersion of CNCs in the polymer matrix [10].

The dispersion quality of CNCs in PHBH is affected by several parameters, including the solvent used during solution casting, the CNC loading, and the structural characteristics of the polymer. However, studies that systematically evaluate the combined influence of these variables on PHBH/CNC nanocomposites are still limited.

The primary objective of this study is to investigate the effect of different solvents employed in the solution casting process on CNC dispersion and to examine how

solution type, CNC content, and CNC type influence the mechanical, thermal, and rheological properties of the nanocomposites. In this work, tetrahydrofuran (THF), chloroform (CHL), dimethylformamide (DMF), and dimethyl sulfoxide (DMSO) were used as solvents to prepare PHBH/CNC films. After identifying the most suitable solvent, nanocomposites with different CNC loadings were produced. Mechanical and thermal analyses were used to assess the applicability of the materials for industrial use, while rheological measurements provided insight into CNC dispersion, viscoelastic behavior, and processability.

2. BIOPLASTICS

2.1 The Importance of Bioplastics and Their Classification

Global plastic production has increased in recent years, reaching around 430 million tonnes in 2024. Most of this production is based on fossil-derived plastics, while recycled and bio-based plastics remain a relatively small part of total production. Approximately 89.9% of plastics are produced from fossil-based resources and are generally non-biodegradable. As a result, the increasing volume of plastic waste has become a significant environmental concern. In contrast, bio-based polymers account for only about 0.6% of total production, a proportion that remains far below the desired amount [13]. Bioplastics represent a promising alternative, as they are biodegradable and/or produced from renewable resources. Biodegradable bioplastics help reduce the amount of plastic waste released into the environment, while bio-based plastics contribute to lowering carbon emissions by reducing dependence on fossil resources. Bioplastics can be classified into three main classes: bio-based and biodegradable plastics, non-biodegradable polymers derived from renewable resources, and biodegradable plastics made from non-biobased sources.

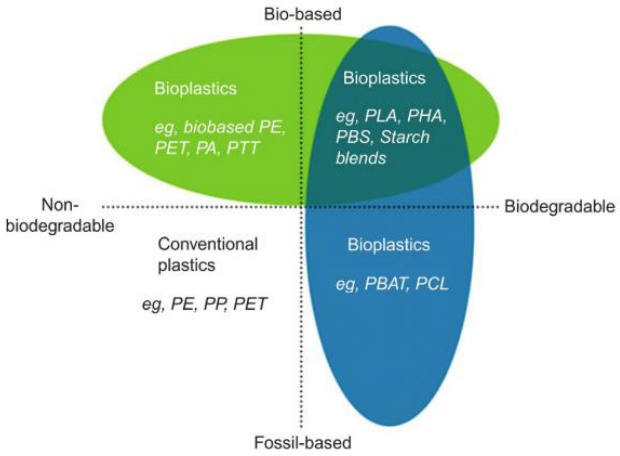

Figure 2.1 : Categorization of plastic materials [12].

Global bibobased plastic production was 2.31 million tonnes in 2025 and is expected to increase to 4.69 million tonnes by 2030 Regarding applications areas, the packaging

sector has the highest share, accounting for 41.3% of the total bioplastics market in 2025, which corresponds to about 0.95 million tonnes [11].

In terms of polymers, polyhydroxyalkanoates (PHAs) show strong growth. PHAs represent 4.7% of total biobased plastic production in 2025, and this share is expected to rise to 16.8% by 2030. This indicates that PHA is the one of the fastest-growing biobased plastics. This growth is mainly linked to the fully biodegradable character of PHAs and the increasing demand for sustainable packaging [11].

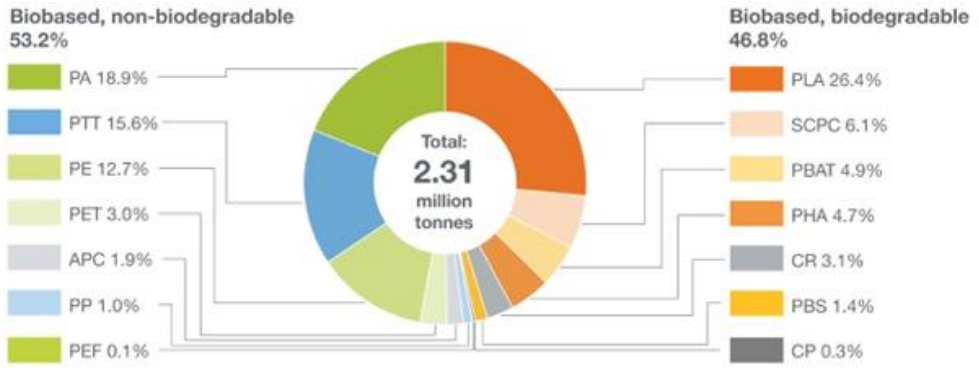

Figure 2.2 : Worldwide production capacity of biobased plastics in 2025 grouped by polymer type [11].

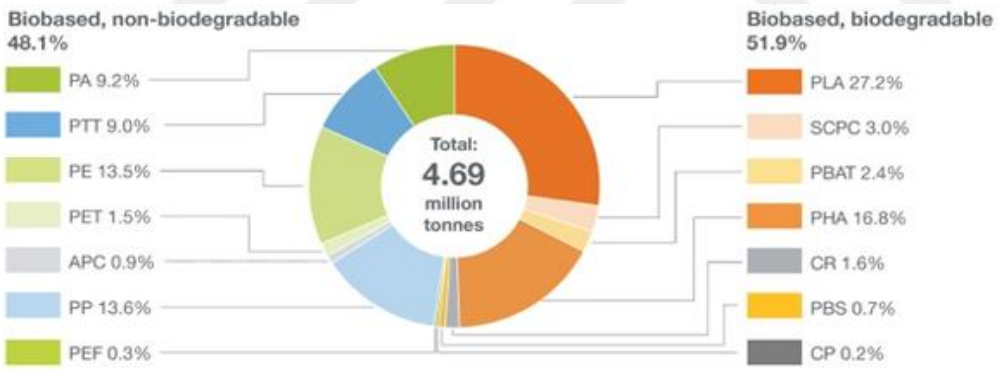

Figure 2.3 : Worldwide production capacity of biobased plastics in 2030 grouped by polymer type [11].

In recent years, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH), a member of the PHA family, has gained increasing attention as a biodegradable polymer and has been used in various industrial applications. One of the main application areas of PHBH is packaging, where it is used for films, bags, and food packaging materials due to its biodegradable and bio-based nature.

PHBH is also applied in agriculture, particularly in biodegradable mulch films that help maintain soil moisture, improve crop growth, and reduce plastic waste in soils. In

addition, PHBH shows potential for marine-degradable products, as it can degrade under natural environmental conditions

Furthermore, PHBH has been investigated for biomedical applications, such as drug delivery systems and biodegradable implants, due to its biocompatibility and biodegradability. These properties make PHBH a promising alternative to conventional petroleum-based plastics in several industrial sectors.

3. LITERATURE REVIEW

3.1 Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH)

Polyhydroxyalkanoates (PHAs) represent a family of aliphatic polyesters produced by microorganisms and are well known for being biobased, biodegradable, and biocompatible. Within this group, poly(3-hydroxybutyrate) (PHB) has received significant industrial interest because of its total biodegradability and pronounced hydrophobic character [14]. However, its practical usage in melt processing is limited as the temperature at which PHB degrades thermal (about 200 °C) is very close to its melting point (around 180 °C), resulting in a narrow processing window and poor thermal stability [15]. For this reason, different modification methods, including polymer blending and copolymerization, have been widely employed to improve the processability. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH) are typical examples of these copolymers. Because it has better mechanical properties, a wider processing temperature range, and greater thermal stability than both PHB and PHBV. Among PHA-based materials, PHBH has gained considerable attention because it offers better thermal stability, a broader processing window, and improved mechanical behavior compared with PHB and PHBV. It is also fully biodegradable and compostable in different environments, including soil, marine conditions, and industrial composting systems, which makes it a promising option for sustainable material development. However, despite these favorable characteristics, the large-scale industrial adoption of PHBH is still restricted. This limitation is mainly associated with its relatively high production cost as well as its weaker rheological and mechanical performance when compared with conventional petroleum-based plastics.

3.1.1 Synthesis and molecular structure

PHBH is a copolymer within the PHA family, and its production depends on the carbon source, environmental conditions, and the microorganism used. The chosen carbon source is metabolized inside the cell and converted into hydroxyacyl-CoA

compounds, which are essential for PHA synthesis. PHA production can occur through one of three pathways: the acetoacetyl-CoA pathway (Pathway I), the de novo fatty acid synthesis pathway (Pathway II), or the β-oxidation pathway (Pathway III), as schematically shown in Figure 3.1. PHAs are polymers composed of repeating 3-hydroxyalkanoic acid units, typically containing 100 to 30,000 monomer units. The R group in the polymer chain varies depending on the microorganism, allowing the formation of different PHA types [15,16]. Table 1 lists common PHA types according to the R group. When the R group is a methyl group (–CH_3), the resulting polymer is poly(3-hydroxybutyrate) (PHB). PHB is widely used in research and industry due to its relatively simple production and highly regular, crystalline structure. Introducing comonomer units into PHB produces copolymers, such as poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH), where 3-hydroxyhexanoate (3HH) units are randomly incorporated along the chain. This reduces chain regularity and allows the composition of the copolymer to be adjusted. Figure 3.2 shows the molecular structures of PHB, PHBH, and PHBV copolymers.

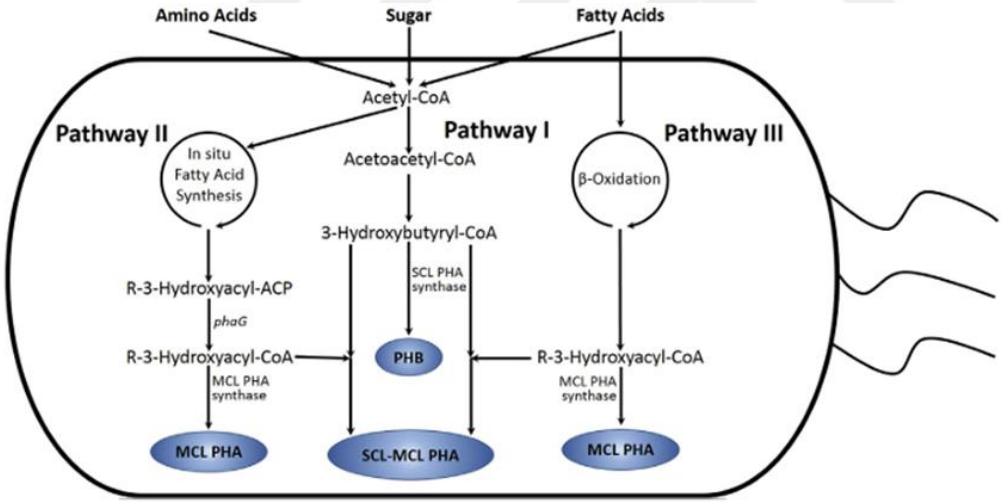

Figure 3.1 : Main biosynthetic routes responsible for PHA formation, including the acetoacetyl-CoA pathway (I), fatty-acid synthesis-based route (II), and the β-oxidation pathway (III) [18].

Table 3.1 : Common R groups and their corresponding polymers [6].

R group type	Polymer Name
Hydrogen (H)	Poly(3-hydroxypropionate) (PHP)
Methyl (-CH_3)	Poly(3-hydroxybutyrate) (PHB)
Ethyl(-C_2H_5)	Poly(3-hydroxyvalerate) (PHV)
Propyl(-C_3H_5)	Poly(3-hydroxyhexanoate) (PHH)
Pentyl(-C_5H_{11})	Poly(3-hydroxyoctanoate) (PHO)

The first observation of PHBH production was reported in 1989, when *Rhodospirillum rubrum* was shown to synthesize this copolymer using butyrate or hexanoate as the carbon source [19]. A few years later, in 1993, the soil bacterium *Aeromonas caviae* FA440 was identified as another microorganism capable of producing a random 3HB–3HH copolymer from substrates such as fatty acids, natural fats, and oils [20].

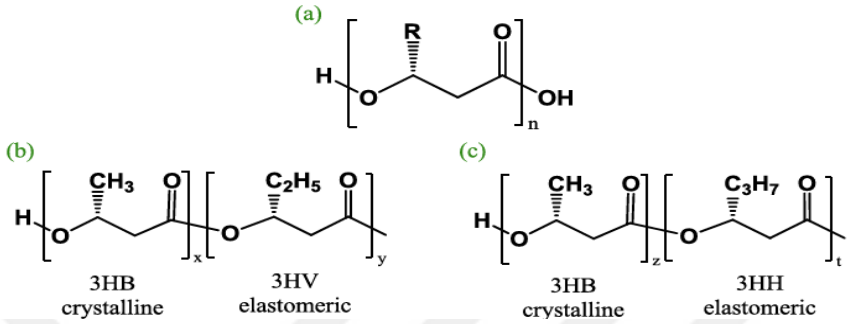

Figure 3.2 : Molecular structures of (a) a generic PHA, (b) PHBV, and (c) PHBH [6].

3.1.2 Mechanical properties

The 3-hydroxyhexanoate (3HH) unit is classified as a medium-chain-length (MCL) monomer because it contains six carbon atoms originating from 3-hydroxyhexanoic acid. Compared with the short methyl side group ($–CH_3$) of the 3HB unit in PHB, 3HH carries a longer propyl side chain ($–C_3H_7$), which strongly affects the molecular architecture of the copolymer [20]. The proportion of 3HH incorporated into PHBH plays a crucial role in determining both the morphology and the mechanical response of the material. As the 3HH content increases, the regular packing of PHB chains is increasingly disordered, which lowers the degree of crystallinity and increases the fraction of amorphous regions within the polymer [6].

This disruption of the crystalline structure leads to a reduction in stiffness-related properties such as tensile strength and Young's modulus. At the same time, greater chain mobility allows the material to undergo larger deformations, resulting in a significant rise in elongation at break. Consequently, PHBH grades with a low 3HH fraction tend to exhibit more rigid and brittle behavior, whereas higher 3HH contents result in materials with greater flexibility and ductility. Although reported mechanical values differ depending on synthesis pathways and copolymer composition, a consistent trend can be observed in the literature: increasing 3HH content generally decreases strength and stiffness while enhancing flexibility and ductility [22].

Figure 3.3 summarizes the effect of 3HH content on the mechanical properties based on the study of Erarslan et al [6]**.** The obtained results are consistent with the literature data. As the 3HH content increases, a decrease in tensile strength and Young's modulus is observed, while an increase in elongation at break occurs. Therefore, in PHBH copolymers, the 3HH content can be regarded as one of the key parameters controlling the transition of the material from a brittle structure to a ductile one. This balance is of critical importance in composition design depending on the desired application (e.g., packaging, films, biomedical applications).

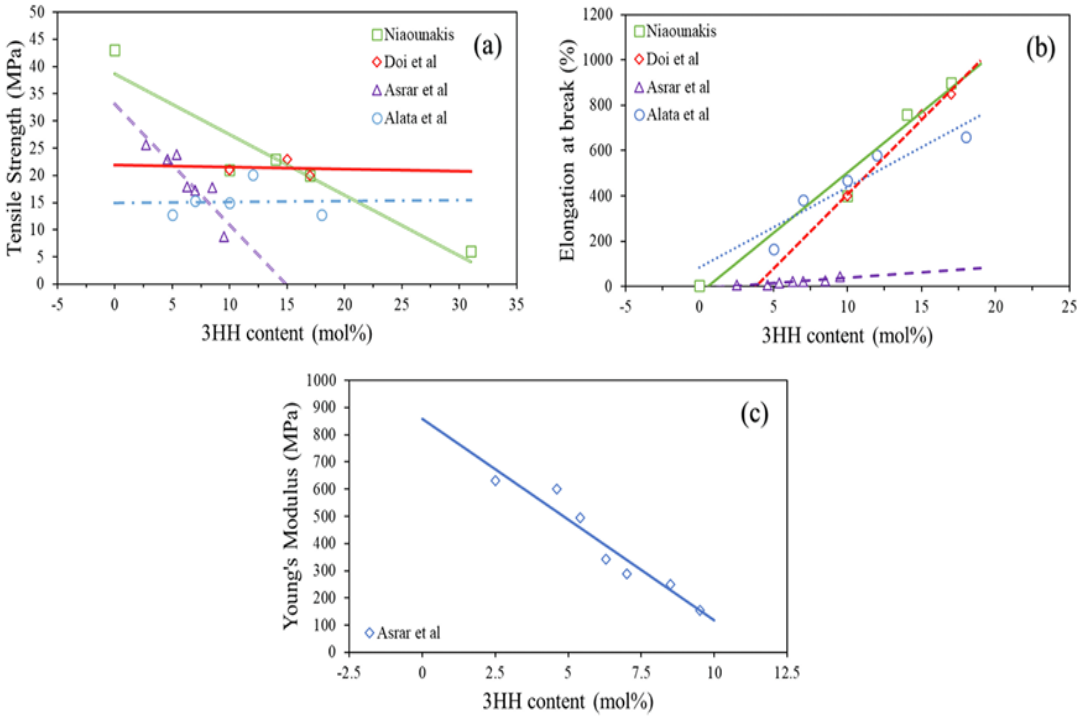

Figure 3.3 : The mechanical behavior of PHBH samples with different 3HH contents, including (a) tensile strength, (b) elongation at break, and (c) elastic modulus [6].

The mechanical performance of the PHA group remains limited compared to petroleum-based polymers. The mechanical propert values of the related polymers are presented in Table 3.2. PHBH, a member of the PHA family exhibits a tensile strength in the range of approximately 15-32 MPa, whereas PET can reach 140-160 MPa, and PP up to 100 MPa. This comparison clearly indicats that PHA groups demonstrate significantly lower mechanical performance than concentional petroleum-based polymers.

Table 3.2 : Mechanical properties of selected polymers [23]**.**

Polymer Type	Tensile Strength (MPa)	Elongation at Break (%)
PET	~140- 160	~60- 180
PVA	~40- 120	~10- 300
HDPE	~18- 32	~20- 60
PHA	~15- 32	~4- 40
PP	~30- 100	~200- 800
LDPE	~7- 25	~250- 1000

3.1.3 Thermal properties and crystallization behavior

The thermal characteristics of PHBH copolymers are highly dependent on the amount of 3-hydroxyhexanoate (3HH) incorporated into the polymer chain. An increase in the 3HH molar fraction generally leads to lower glass transition temperature (Tg) and melting temperature (Tm), together with a marked reduction in crystallinity.

Increasing the 3HH content in PHBH enhances the flexibility of the polymer chains, which shifts the glass transition temperature (Tg) to lower values. Experimental studies have shown that a 10 mol% increase in 3HH typically reduces Tg by 2–3 °C [20,24], while another study reported a slightly larger decrease of 3–4 °C for the same increase in comonomer content [25]. These results suggest that the incorporation of 3HH disrupts the regular packing of polymer chains and increases the amorphous character of the copolymer.

The effect of 3HH on melting behavior is even more pronounced. Each additional 10 mol% of 3HH in the copolymer has been found to lower the melting temperature by approximately 15–25 °C [20], reflecting a significant reduction in crystalline order due to the larger 3HH side groups. As shown in Figure 3.4, increasing the 3HH content also raises the onset temperature of degradation, based on the findings reported by Asrar et al. [26].

Asrar et al. investigated the effect of 3HH content on the crystallization half-time. As shown in Figure 3.5**,** an overall increase in crystallization half-time is observed with increasing 3HH content[26]. This suggests that the slow crystallization behavior becomes more noticeable as the 3HH fraction increas and that crystal formation in PHBH takes place over increasingly longer periods.

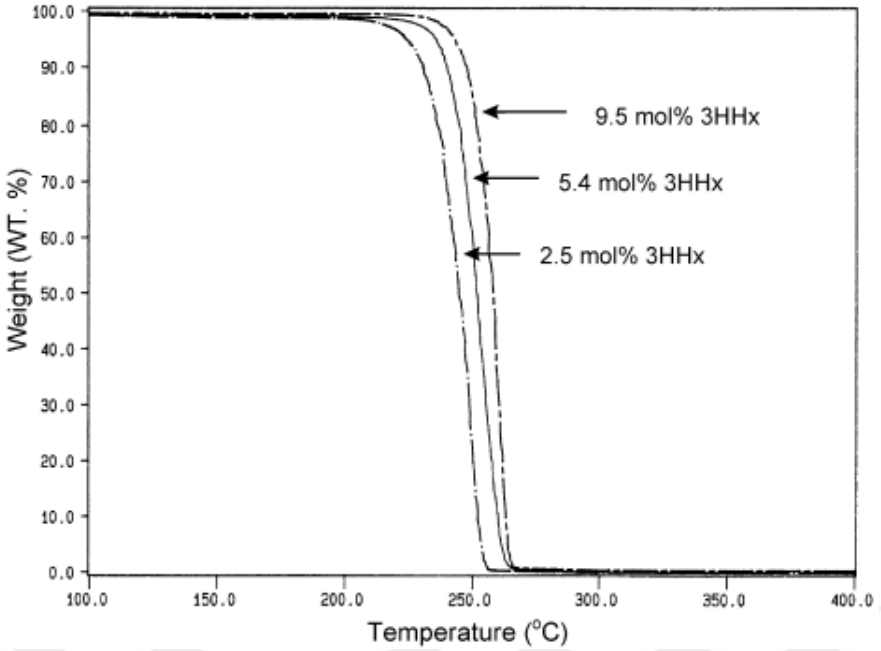

Figure 3.4 : Thermal degradation profiles showing mass loss as a function of temperature for poly(3HB-co-3HHx) films containing 2.5, 5.4, and 9.5 mol% of 3HH [26].

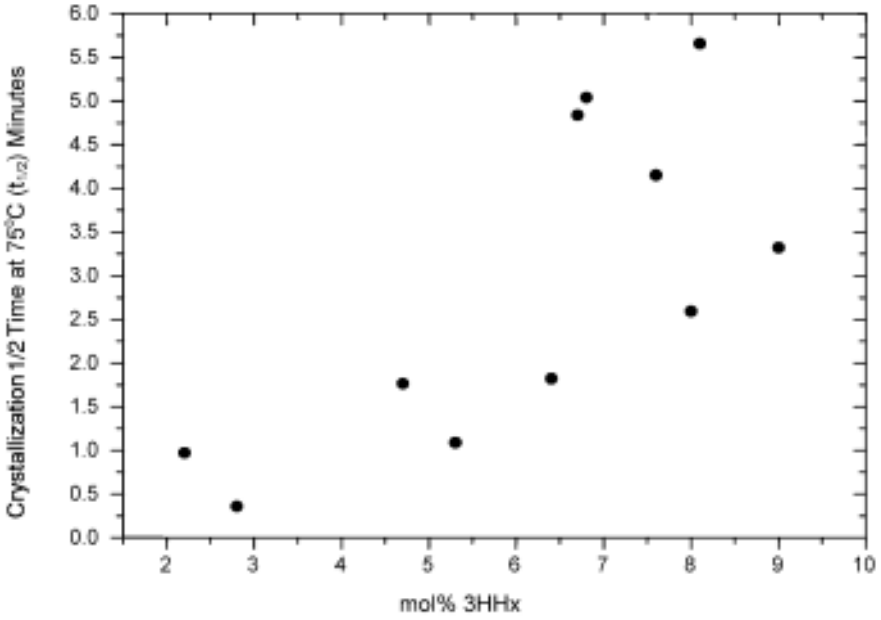

Figure 3.5 : Dependence of crystallization half-time ($t_{1/2}$) on the 3HH molar content [26].

3.1.4 Rheological behavior

Rheological information is essential for understanding the melt-state behavior of polymers and composite structures and, consequently, for predicting their processability[27]. In addition, rheological analyses provide helpful information into the effects of added particles in composite systems, offering valuable results regarding

filler dispersion, percolation structure, and matrix–filler interfacial interactions. When neat PHBH is considered, its processability, formability, and foamability are highly limited due to slow crystallization and low melt strength. The rheological behavior of PHBH copolymers is dependent on temperature, molecular weight distribution, and 3HH content.

Rheological studies on PHBH are still limited in the literature. It is well known that neat PHBH exhibits a typical shear-thinning behavior characteristic of polymer melts[28]. The effect of increasing 3HH content on the rheological properties is presented in Figure 3.6 and Figure 3.7. According to the graphs shown in this figure, a significant decrease in the storage modulus (G′) is observed with increasing 3HH content. This behavior shows that the material loses its elastic modulus and deviates from solid-like behavior.

Moreover, according to Figure 10, at low 3HH contents, the range over which te storage modulus (G′) dominates the loss modulus (G″) is relatively broad. However, as the 3HH content increases, G″ becomes significantly more dominant than G′ and exhibits higher values over almost the entire range. This behavior indicates a transition of the material from solid-like to liquid-like behavior.

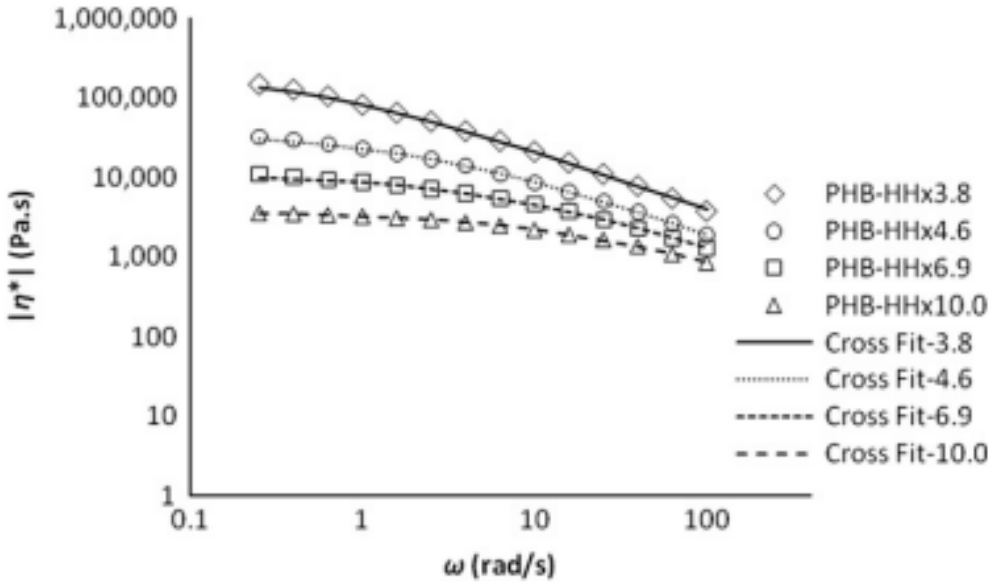

Figure 3.6 : Complex shear viscosity curves of PHBH with different 3HH content [28].

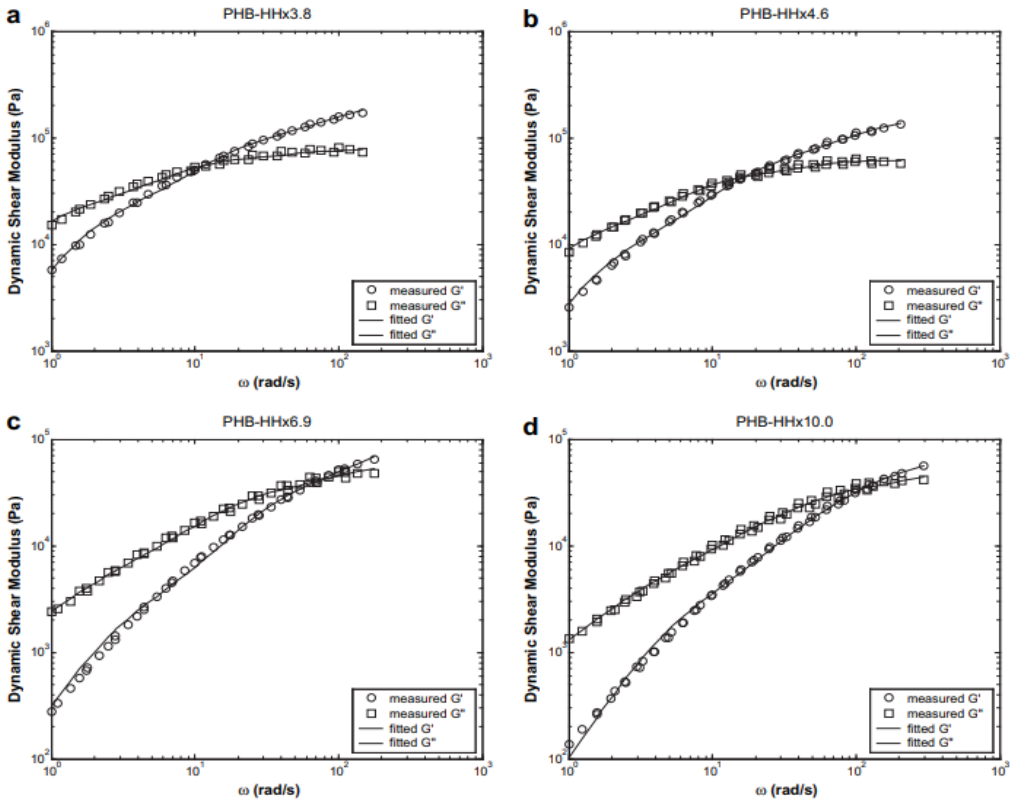

Figure 3.7 : The Generalized Maxwell model was used to describe the dynamic shear behavior of PHB-HHx [28].

Changing temperatures have a big impact on rheological behavior because polymers are more sensitive to thermal degradation as the temperature rises. According to Nofar et al.[29], the PHBH X151C copolymer is highly prone to thermal degradation in the molten state at temperatures above 160 °C. As shown in Figure 3.8, while a slight improvement in viscosity is observed at low frequencies at 160 °C, a significant decrease in viscosity occurs in the low-frequency region with further increases in temperature. This behavior clearly indicates that PHBH undergoes significant thermal degradation in the melt at elevated temperatures.

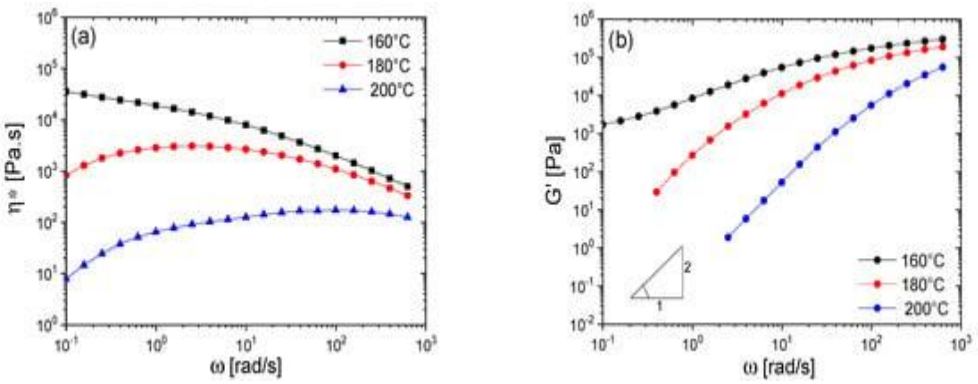

Figure 3.8 : Complex viscosity (a) and storage modulus (b) of PHBH X151C measured at different temperatures [29].

In the study conducted by Liao et al. [28], the time-dependent development of complex viscosity for PHB-HHx4.6 was investigated at temperatures of 140, 150, and 160 °C. With increasing temperature, a decrease in viscosity was observed over time, which explained by thermal degradation occurring in the molten state. In particular, compared to the other two temperatures, a significantly more noticeable decrease in complex viscosity was found at 160 °C, indicating that the PHB-HHx copolymer becomes thermally unstable at high temperatures.

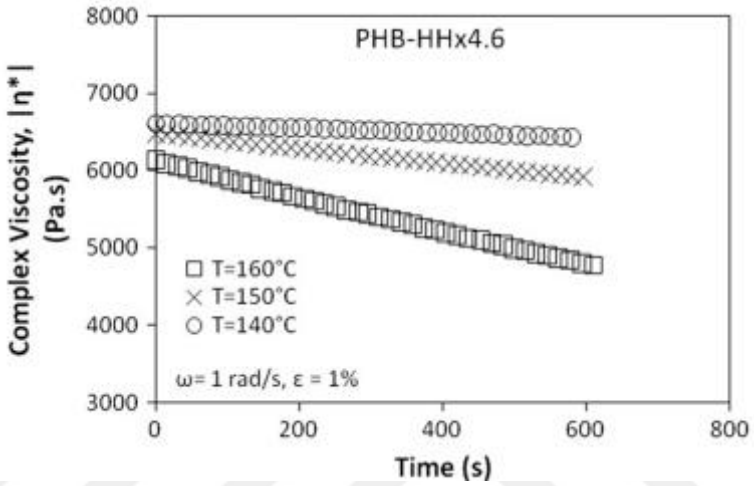

Figure 3.9 : Time-dependent complex shear viscosity of PHB-HH under oscillatory shear [28].

3.1.5 Degradation behavior

Biodegradable polymers can be composted in various environments; however, the PHB family exhibits significantly superior compostability behavior**.** One of the main reasons PHB-based polymers are considered strong alternatives to petroleum-based plastics is their ability to be composted in a wide range of environments. Although they are still slightly inferior in terms of mechanical, thermal, and rheological properties, their excellent degradation behavior makes them a very powerful alternative.

Morse et al. [30] reported that the biodegradation rate increases with increasing 3HH content**.** In contrast, Wang et al. [31] argued that there is a critical threshold**,** showing that when the 3HH content exceeds 15%**,** the biodegradation rate of PHBH starts to decrease**.**

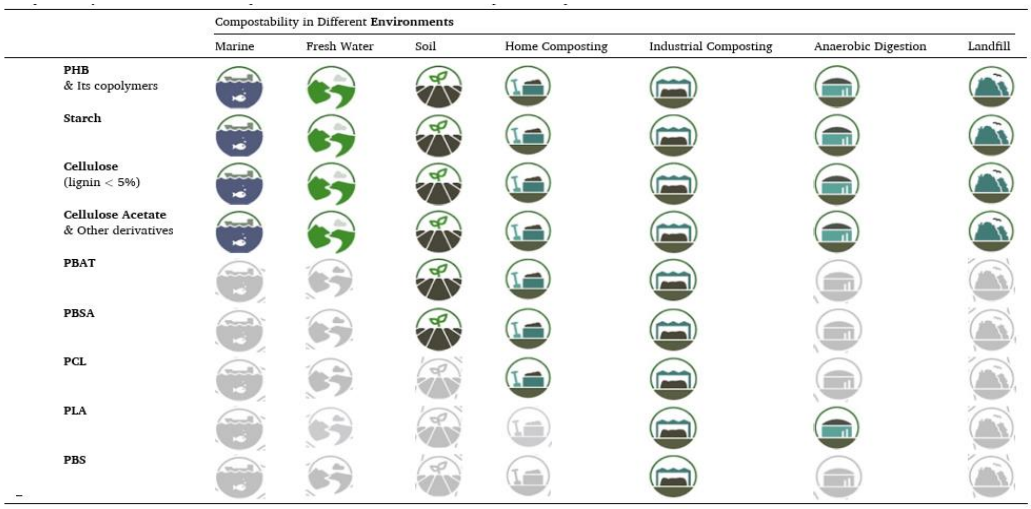

Figure 3.10 : Composting behavior of different bioplastics under various environmental conditions [6].

3.1.6 Efforts to improve properties of PHBH

PHBH is a biobased and biodegradable polymer with excellent compostability. Despite these advantages, its application potential in packaging, textile, medical, and automotive sectors is limited by several intrinsic drawbacks. In particular, its low mechanical strength, slow crystallization rate, and poor rheological properties hinder its widespread use in industrial applications. Therefore, various methods have been developed to improve the mechanical and rheological properties of PHBH and to make it competitive with petroleum-based polymers. These methods include copolymerization, the addition of plasticizers, polymer blending, and making composites or nanocomposites. Each method offers various advantages, and the choice of method depends on the specific application and required properties. In recent years, nanocomposite systems incorporating nanoparticles have attracted increasing attention due to their ability to provide significant performance enhancements even at low filler contents.

3.2 Cellulose Nanocrystals (CNCs)

Nanocellulose consists of cellulose-based materials with nanoscale dimensions, produced from natural cellulose through treatments such as acid hydrolysis and related processes [32]. Based on their structure and method of preparation, nanocellulose is commonly divided into three main types: cellulose nanocrystals (CNCs), cellulose nanofibrils (CNFs), and bacterial nanocellulose (BNC). CNCs mainly contain highly crystalline regions that remain after the amorphous parts of cellulose are removed

during acid hydrolysis. In contrast, CNFs have a fibrous structure and include both crystalline and amorphous domains [33]. BNC is produced by certain bacteria and is distinguished by its high purity and three-dimensional nanofibrous network.

All types of nanocellulose possess abundant hydroxyl (–OH) groups on their surfaces, and depending on the hydrolysis conditions, additional negatively charged groups such as sulfate or phosphate functionalities may also be present. These surface chemistries are responsible for the strong interaction of nanocellulose with water and its highly hydrophilic nature [34].

Although the exact dimensions and crystallinity of CNCs vary with the cellulose source and processing method, their typical diameters are in the range of 5–10 nm, while their lengths commonly extend from tens to several hundreds of nanometers [35]. CNCs exhibit outstanding properties, including very high crystallinity (often exceeding 85%), tensile strength in the range of 2–3 GPa, large specific surface area, extremely low thermal expansion, high aspect ratio, and elastic moduli on the order of 110–220 GPa. These features make them highly attractive as reinforcing nanofillers in advanced and multifunctional composite materials [36,37]. At the same time, the high density of surface hydroxyl groups promotes strong hydrogen bonding between CNC particles, which contributes to their pronounced hydrophilicity.

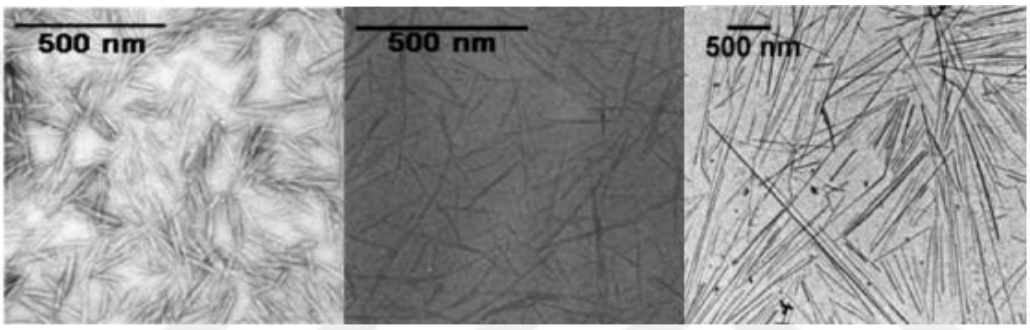

Figure 3.11 : TEM images of CNC suspensions obtained from cotton (left), sugar-beet pulp (middle), and tunicin (right) [38].

3.3 PHBH/CNC Nanocomposites

Growing environmental awareness and the sustainability challenges associated with fossil-based plastics have accelerated the search for biodegradable and bio-based polymer alternatives. Within this framework, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH), which belongs to the polyhydroxyalkanoate (PHA) family, has gained increasing interest because it is derived from renewable resources

and is fully biodegradable. Nevertheless, its relatively low mechanical performance, limited thermal resistance, and insufficient rheological behavior still hinder its broader use in engineering-level applications.

To address these drawbacks, cellulose nanocrystals (CNCs) have been widely investigated as reinforcing agents for PHBH, owing to their high stiffness, large specific surface area, and sustainable origin [39]. When CNCs are well dispersed and strong interfacial interactions are established, PHBH/CNC nanocomposites can display notable enhancements in mechanical strength, thermal stability, and viscoelastic performance.

The influence of nanoparticles on the mechanical properties of PHBH mainly depends on achieving uniform dispersion within the polymer matrix and strong interfacial bonding. These factors are essential to avoid nanoparticle aggregation, which can weaken the polymer structure and reduce both tensile strength and elongation at break. When CNCs are homogeneously distributed and interact effectively with the matrix, a clear improvement in mechanical performance is obtained.

Li et al. [40] reported that rigid nanocrystals can significantly increase the elastic modulus of polymer systems, while excessive filler loading may cause particle agglomeration and restrict chain mobility, leading to a decline in mechanical properties. In addition, the decrease in elongation at break is commonly related to the reduced flexibility of polymer chains in the presence of nanocrystals.

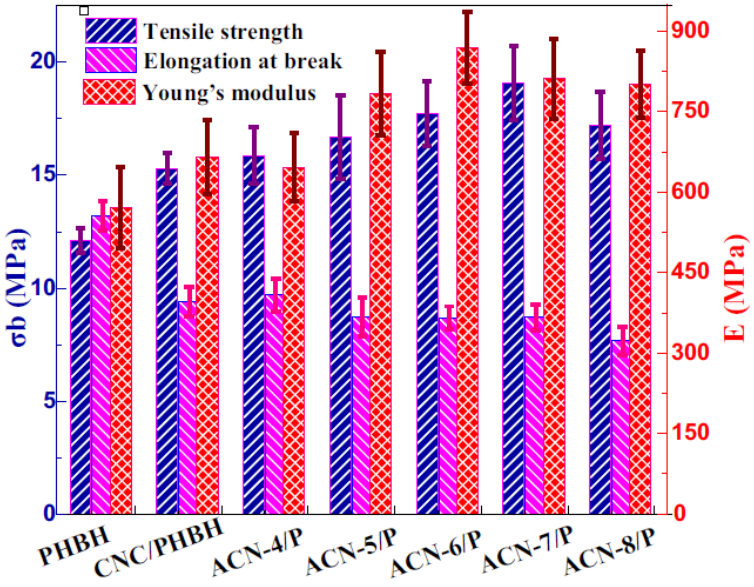

Figure 3.12 : Mechanical performance of neat PHBH and PHBH-based nanocomposites reinforced with CNC and ACN [40].

Tensile strength generally increased with increasing CNC content, reaching a maximum value of 19.05 MPa for the ACN-7/P sample. In contrast, a reduction in tensile strength was observed for ACN-8/P, which can be attributed to the excessive addition of CNCs that leads to poor dispersion and the formation of agglomerates. A similar trend was found for Young's modulus, with the highest value of 869 MPa also obtained for ACN-7/P.

Meanwhile, the elongation at break decreased as the CNC content increased. This reduction is related to the presence of rigid CNC particles, which limit the mobility of the polymer chains. Strong interfacial interactions and the formation of physical networks restrict chain deformation, resulting in a more brittle mechanical behavior.

Hosoda et al. [41] examined the mechanical performance of PHBH/CNC nanocomposites, and their findings are consistent with trends reported in previous studies. The incorporation of CNCs led to a marked improvement in stiffness compared with neat PHBH. Specifically, the Young's modulus increased from 271 MPa for the unfilled polymer to 378 MPa with 1 wt% CNC and reached 746 MPa at a CNC loading of 4 wt%. A similar enhancement was observed for tensile strength: while neat PHBH exhibited a value of approximately 15 MPa, this increased to about 20 MPa for PHBH containing 1 wt% CNC and to roughly 27 MPa for the 4 wt% CNC formulation. On the other hand, ductility decreased as the CNC content increased. The elongation at break dropped from around 20% for neat PHBH to nearly 8% for the composite with 4 wt% CNC.

Neat PHBH also shows favorable thermal degradation characteristics. The thermogravimetric (TG) curves of PHBH and its CNC-filled nanocomposites are presented in Figure 3.13. The addition of CNCs results in a noticeable shift of the degradation temperature to higher values compared with the neat polymer. Moreover, the temperature corresponding to the completion of degradation increases almost linearly as the CNC content rises. This behavior is attributed to strong interfacial interactions between the PHBH matrix and the CNC particles, which suggests that the nanofillers are well dispersed within the polymer system.

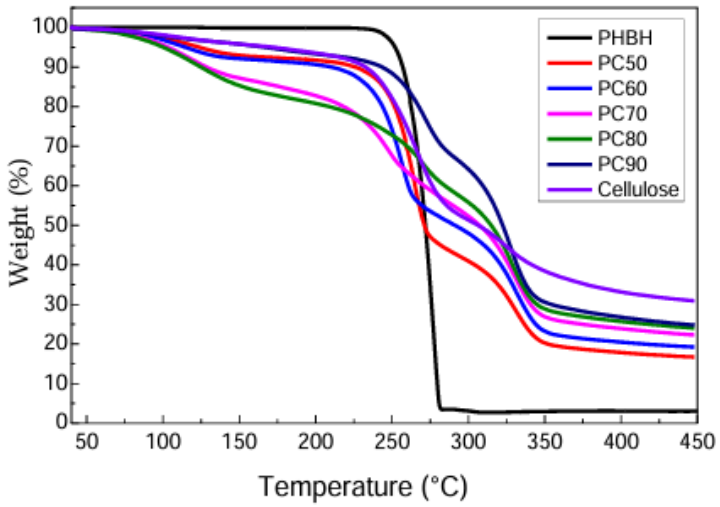

Figure 3.13 : TG curves of PHBH/cellulose composites [42].

PHAs need to be further developed in order to be competitive with other petroleum-based polymers. CNC reinforcement is very important for this purpose**,** because if CNC dispersion is achieved effectively, a significant improvement in the barrier properties of the composite can be observed. According to the study of Zhou et al.**,** in PHBH/CNC composites**,** the oxygen permeability decreases with increasing cellulose content**.**

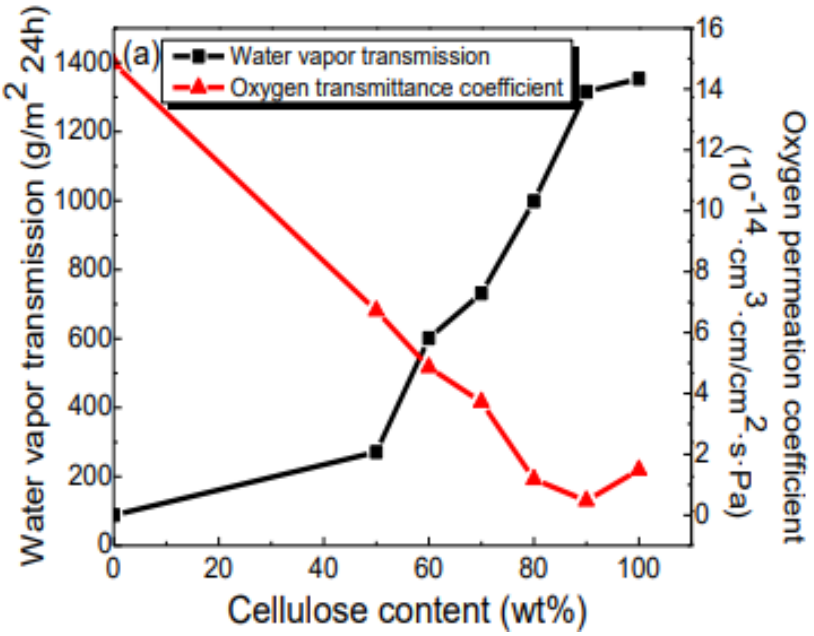

Figure 3.14 : Water vapor transmission rate and oxygen permeation coefficient vs cellulose percentage [43].

4. EXPERIMENTAL PROCEDURE

4.1 Materials

The PHBH used in this study was supplied by KANEKA (grade X331A) and contained 5 mol% of 3-hydroxyhexanoate (3HH), with a melt flow rate of 12 g/10 min determined at 165 °C. This grade was chosen because its relatively high MFI indicates a lower molecular weight, which facilitates better dispersion of nanoparticles within the polymer matrix. Cellulose nanocrystals (CNCs) were obtained from CelluForce (Canada). Tetrahydrofuran (THF, 99%), chloroform (CHL), dimethylformamide (DMF, 99%), and dimethyl sulfoxide (DMSO, 99%) were used as solvents and were purchased from Sigma-Aldrich.

4.2 Preparation of PHBH/CNC Nanocomposites

The procedures followed to prepare PHBH/CNC nanocomposites are summarized in Table 4.2. To study the role of solvent selection, formulations containing 3 wt.% CNC were produced using four different solvents. First, CNC was introduced into each solvent and dispersed by bath ultrasonication for 2 hours. PHBH331A was subsequently incorporated, and the resulting suspensions were magnetically mixed until a homogeneous solution was formed, which typically took about 2.5 hours, while DMSO-based systems required approximately 4 hours.

The temperature applied during stirring depended on the solvent type: THF and chloroform were processed at 40 °C, whereas DMF and DMSO required elevated temperatures of 60 °C and 80 °C, respectively. Once complete dissolution was achieved, the solutions were cast into Petri dishes and left to dry at room temperature for two days. The samples were then further dried under vacuum at 85 °C for an additional two days, with DMSO-containing samples undergoing extended vacuum drying for up to five days.

After solvent removal, the resulting films were ground into fine powders using a coffee grinder. These powders were vacuum-dried overnight at 55 °C prior to compression

molding. Rheological test specimens were subsequently fabricated following the conditions given in Table 4.1, by applying a pressure of 1.5 tons at 160 °C for 5 minutes.

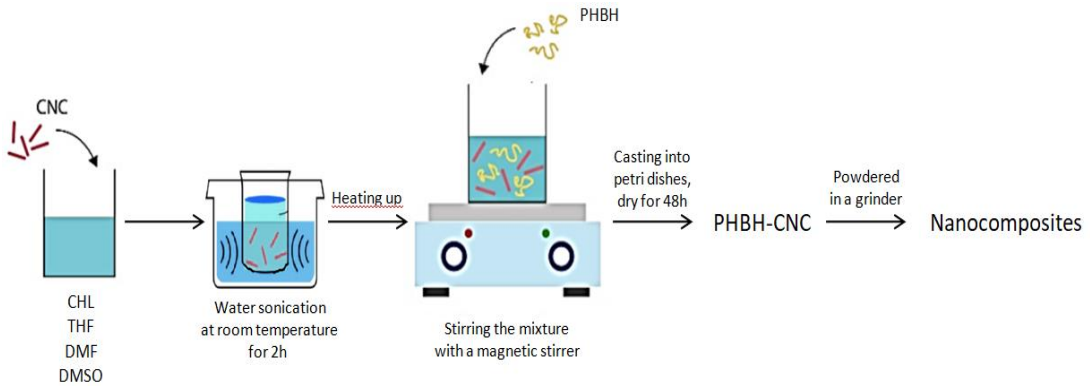

Figure 4.1 : Schematic illustration of the preparation of PHBH/CNC nanocomposites by the solvent casting method.

Once the best solvent for CNC dispersion was found, the effect of CNC concentration was studied by making PHBH/CNC nanocomposites with varying filling amounts (0, 0.1, 1, 3, and 5 wt.%) while using DMSO as the solvent. These mixtures were made using the best temperature and processing time settings that had already been found for DMSO. They were then ready to be characterized and tested.

Table 4.1 : Summarized the preparation processes of PHBH/CNC nanocomposites.

	Solvent	Preparation	Temperature		Drying
1	CHL	CNC %3 + CHL PHBH 331A + CNC %3 CHL Mixture	WS, 2h MS, 2.5h	RT 40 ^{0}C	
2	THF	CNC %3 + THF PHBH 331A + CNC %3 THF Mixture	WS, 2h MS, 2.5h	RT 40 ^{0}C	
3	DMF	CNC %3 + DMF PHBH 331A + CNC %3 DMF Mixture	WS, 2h MS, 2.5h	RT 60 ^{0}C	Drying under fume hume
4	DMSO	CNC %3 + DMSO PHBH 331A + CNC %3 DMSO Mixture	WS, 2h MS, 4h	RT 80 ^{0}C	(RT, 2D) Vacuum drying
5	DMSO	No CNC + DMSO PHBH 331A + No CNC DMSO Mixture	WS, 2h MS, 4h	RT 80 ^{0}C	of film (85 ^{0}C, 2D), For DMSO Vacuum
6	DMSO	CNC %0.1 + DMSO PHBH 331A + CNC %0.1 DMSO Mixture	WS, 2h MS, 4h	RT 80 ^{0}C	drying of film (85 ^{0}C, 5D),
7	DMSO	CNC %1 + DMSO PHBH 331A + CNC %3 DMSO Mixture	WS, 2h MS, 4h	RT 80 ^{0}C	Vacuum drying of powder (85 ^{0}C, 2D)
8	DMSO	CNC %3 + DMSO PHBH 331A + CNC %3 DMSO Mixture	WS, 2h MS, 4h	RT 80 ^{0}C	
9	DMSO	CNC %5 + DMSO PHBH 331A + CNC %5 DMSO Mixture	WS, 2h MS, 4h	RT 80 ^{0}C	

4.3 Characterization

4.3.1 FTIR analysis

Fourier transform infrared (FTIR) spectra of neat PHBH samples prepared using different solvents were recorded in the mid-infrared range (400–4000 cm^{-1}) using a Bruker spectrometer equipped with a Platinum ATR accessory, with all measurements carried out at room temperature.

4.3.2 Differential scanning calorimetry (DSC) analysis

The thermal and crystallization behavior of PHBH/CNC specimens was examined by differential scanning calorimetry (DSC). Measurements were carried out using a TA Instruments Q1000 DSC under a nitrogen atmosphere. Each sample was initially heated from ambient temperature to 200 °C at a rate of 10 °C min^{-1} and held at this temperature for 5 minutes to remove any previous thermal history. The samples were then cooled to −20 °C at the same rate, followed by a second heating scan up to 200 °C at 10 °C min^{-1}.

4.3.3 Rheological analysis

Rheological analyses wew conducted to investigate the CNC disperion on the matrix and viscoelastic behavior of nanocomposites. Frequency and time sweep rheology analyzes were performed with Anton Paar MCR302 rheometer device under the nitrogen atmosphere and frequency sweep analyzes were performed at 160 °C , 1% strain.

5. RESULTS AND DISCUSSIONS

The rheological behavior of PHBH/CNC nanocomposites was studied to understand how adding CNCs affects the viscoelastic properties of PHBH and its processability. Rheological measurements were used to investigate polymer–filler interactions and to examine how well the CNCs were dispersed within the polymer matrix. In addition, the non-isothermal crystallization behavior of the nanocomposites was examined to investigate the effects of CNC loading, solvent type, and CNC content on crystallization kinetics. Differential scanning calorimetry (DSC) was used to determine crystallization temperatures, crystallization rates, and the degree of crystallinity. Overall, these results highlight the nucleating effect of CNCs and show how different processing conditions influence the structural development of PHBH during cooling, which ultimately affects both its processability and final performance.

5.1 The Effect of Solvent

To evaluate how solvent choice influences CNC dispersion, PHBH/CNC nanocomposites containing 3 wt.% CNC were prepared using four different solvent systems. The complex viscosity responses of neat PHBH and the corresponding nanocomposites are compared in Figure 5.1.

For samples produced using chloroform (CHL), which is an apolar solvent with a low dielectric constant, limited CNC dispersion was anticipated. This expectation is confirmed by the rheological curves in Figure 5.1, where no noticeable increase in complex viscosity is observed in the low-frequency region. This result indicates that the CNC particles remained agglomerated within the PHBH matrix, resulting in mainly Newtonian-like behavior.

A similar trend is observed for nanocomposites prepared with tetrahydrofuran (THF). Although THF exhibits some polarity, its relatively low dielectric constant restricts its ability to effectively separate CNC aggregates, resulting again in negligible viscosity enhancement at low frequencies.

In contrast, the nanocomposites obtained using DMF and DMSO display significantly higher complex viscosity values compared to neat PHBH. A significant increase in viscosity at low frequencies indicates the development of non-Newtonian behavior, which is typically associated with improved dispersion and network formation of CNCs within the polymer matrix. In particular, DMSO, owing to its high polarity and dielectric constant, effectively suppresses CNC agglomeration and promotes a more uniform distribution of nanocrystals throughout the composite.

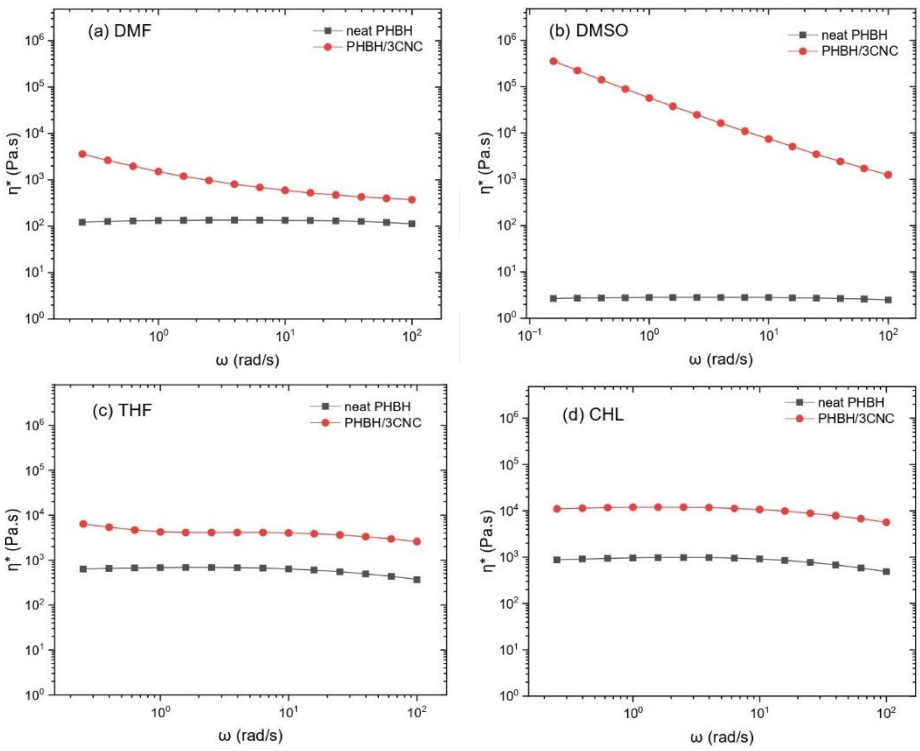

Figure 5.1 : Frequency-dependent complex viscosity of neat PHBH and its nanocomposites prepared using (a) DMF, (b) DMSO, (c) THF, and (d) CHL.

The appearance of the nanocomposites processed with different solvents is shown in Figure 5.2. Samples prepared with DMF and DMSO exhibit a darker coloration, which is commonly associated with a more homogeneous CNC dispersion within the PHBH matrix. It can be stated that the darker appearance of the nanocomposites (NCs) does not originate from the thermal degradation of PHBH or CNC, but rather from the dispersion of CNC within the polymer matrix.

The darker appearance observed particularly in the samples prepared with DMSO may be attributed to the high polarity and high dielectric constant of DMSO. These properties enable stronger interactions with CNC and promote a more homogeneous

and finer dispersion of CNC within the matrix. A more homogeneous and compact structure reduces light scattering, resulting in a darker appearance of the samples.

In contrast, the lower polarity and faster solvent evaporation in CHL and THF systems may lead to a more heterogeneous structure. This situation would create the opposite effect.

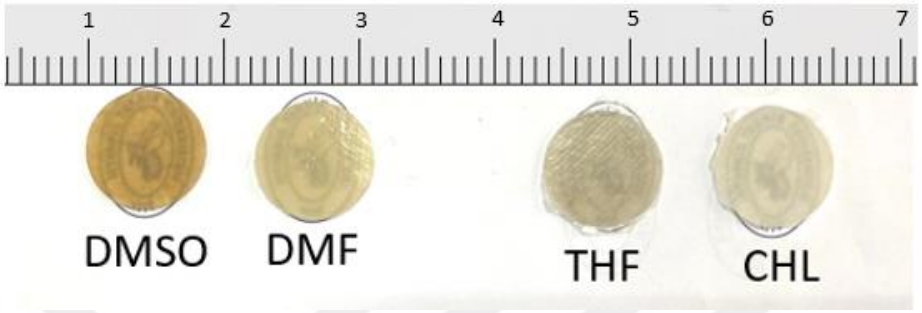

Figure 5.2 : Appearance of PHBH/CNC nanocomposites obtained with different solvent systems.

To further highlight the influence of solvent selection, normalized complex viscosity values are presented in Figure 5.3. The normalization was performed relative to the complex viscosity of the corresponding no CNC-PHBH samples (η*(wo CNC)). A notable increase in normalized viscosity is clearly observed for nanocomposites produced in DMSO, demonstrating the strong reinforcing effect resulting from improved CNC dispersion.

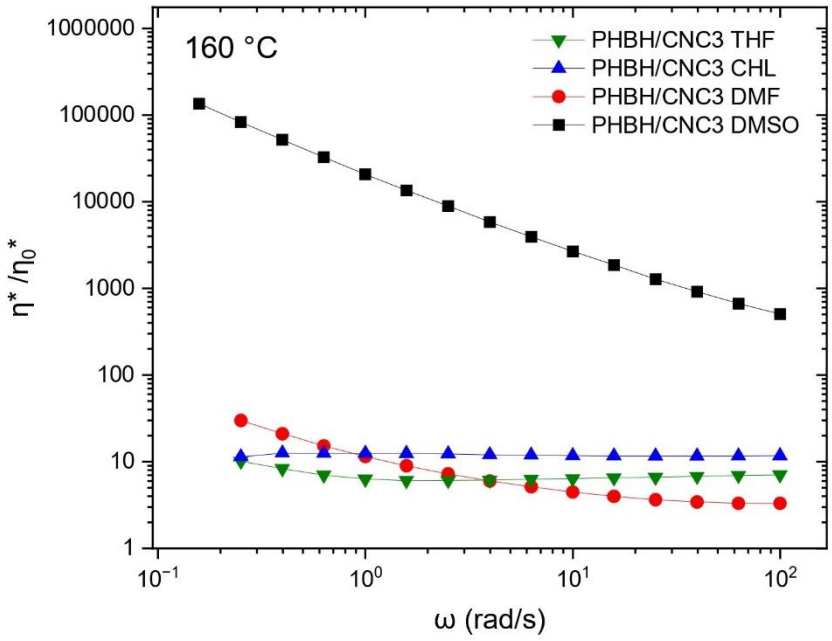

Figure 5.3 : Normalized complex viscosity of PHBH/CNC nanocomposites with 3wt% processed in various solvents at 160 °C.

Samples prepared with DMF show only a minor enhancement, while those processed with CHL and THF do not exhibit any significant change in viscosity. These results confirm that DMSO provides the most effective environment for achieving a well-dispersed CNC network within PHBH.

5.2 Rheological Properties and FTIR Analysis of Neat PHBH

Figure 5.4 compares the complex viscosity of unprocessed PHBH with that of neat PHBH samples prepared using different solvents. In all cases, solvent-treated PHBH exhibits lower viscosity than the original polymer, suggesting that exposure to the solvents may have altered the polymer chain structure or reduced the effective molecular weight. DMSO and DMF have high boiling points (189 and 153 °C, respectively), which makes their complete removal from the films difficult. Since CHL and THF have lower boiling points (61 and 66 °C), the samples prepared with these solvents exhibit rheological behavior closer to that of the untreated polymer in terms of viscosity.

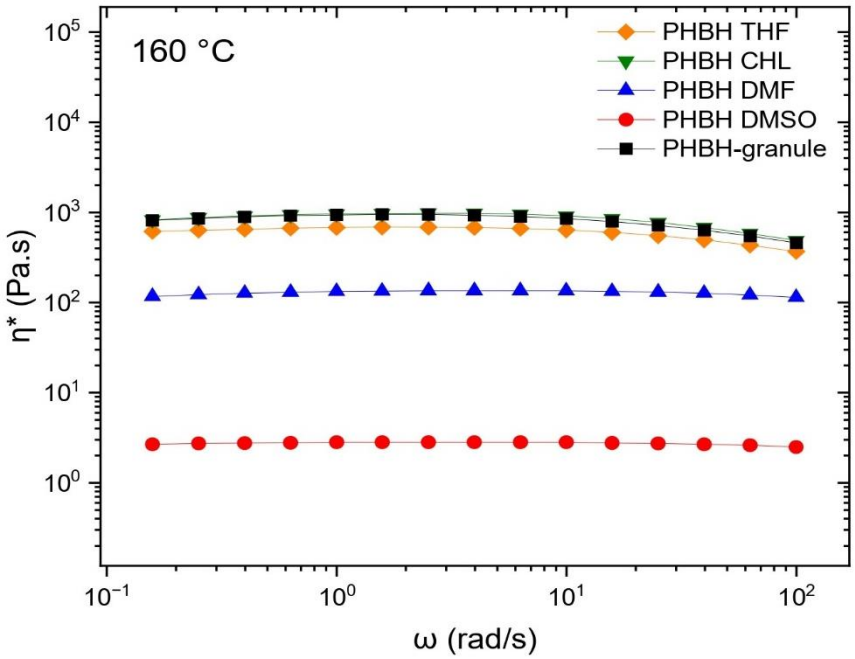

Figure 5.4 : Complex of complex viscosity between unprocessed PHBH and solvent-treated neat PHBH samples.

To investigate whether chemical changes occurred during processing, FTIR analyses were performed, as presented in Figures 5.5 and 5.6. The peaks observed at 2,849, 2,921, and 2,976 cm^{-1} were assigned to the C–H stretching vibrations of the $–CH_3$ groups. In samples prepared with different solvents, a decrease in the intensity of the

peaks at 2,976, 2,932, and 2,873 cm^{-1} was observed. Furthermore, in the sample prepared with DMSO, the weak peak at 2,849 cm^{-1} almost completely disappeared. The decrease in the intensity of the C–H stretching vibrations and the slight shifts in wavenumber indicate that solvent molecules interact with the PHBH chains and alter their chain conformation. This suggests that the solvent may not have been completely removed, or that the chain regularity decreased after processing.

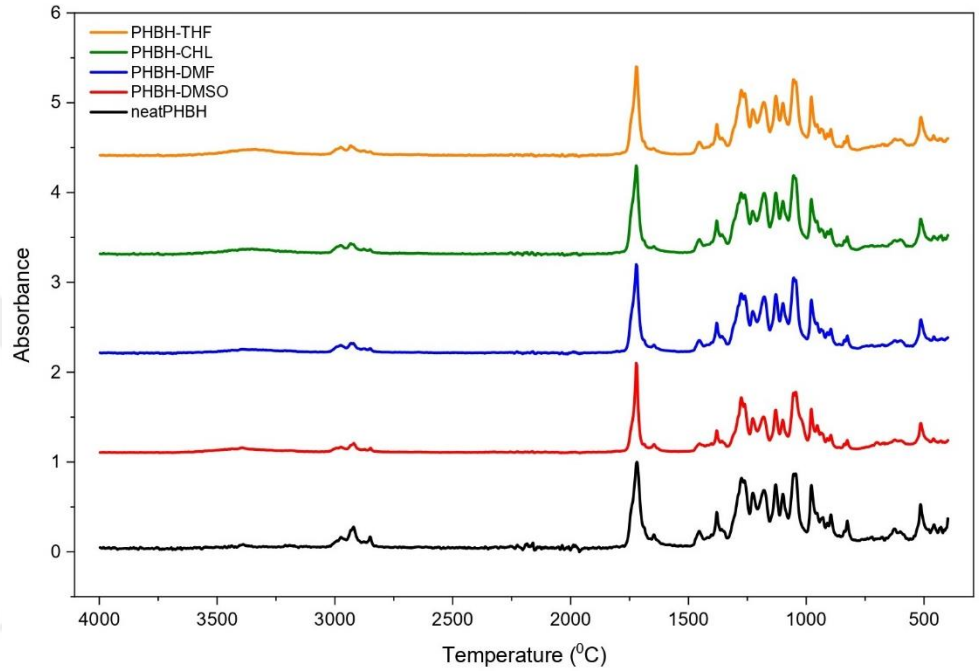

Figure 5.5 : FTIR spectra of neat PHBH samples prepared using different solvents.

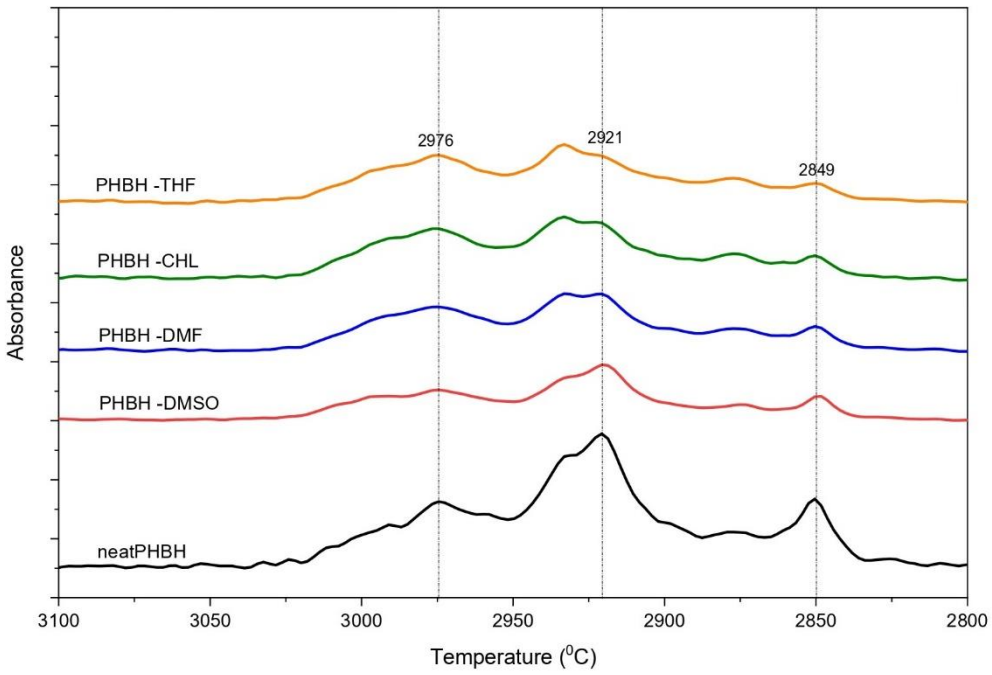

Figure 5.6 : FTIR spectra of neat PHBH in the wavenumber range of 2800–3100 cm^{-1}.

5.3 Investigation of CNC Dispersion and Percolation Threshold

Since PHBH/CNC nanocomposites produced in DMSO showed the most pronounced rheological improvements, this solvent was selected to examine the influence of CNC concentration. Accordingly, nanocomposites containing 0, 0.1, 1, 3, and 5 wt.% CNC were prepared in DMSO. Rheological measurements were then carried out to assess how increasing CNC content affects the viscoelastic response of the system and to identify the percolation threshold.

5.3.1 Frequency sweep results

A rise in complex viscosity at low frequencies is typically related to stronger interactions between CNC particles and the formation of a network within the polymer. In contrast, a flat or slightly decreasing viscosity, which reflects Newtonian behavior, indicates that CNCs are poorly dispersed or remain in agglomerated form. Therefore, a Newtonian response suggests weak nanoparticle dispersion.

Figure 5.7 presents the complex viscosity as a function of frequency for PHBH/CNC nanocomposites containing 0.1, 1, 3, and 5 wt.% CNC prepared in DMSO. All samples show a pronounced increase in viscosity in the low-frequency region, with no distinct plateau, which is a clear sign of network formation. This effect becomes stronger as the CNC content increases. Even at 0.1 wt.% CNC, a noticeable viscosity enhancement is observed, indicating the high effectiveness of DMSO in dispersing CNCs. The strong low-frequency response suggests that the percolation threshold for these nanocomposites is between 0 and 0.1 wt.% CNC.

Figure 5.8 illustrates how the storage modulus (G') changes with frequency for the nanocomposite samples. For neat PHBH, the log–log plot of G' versus frequency shows a slope close to 2, which is typical of polymer melts. When CNCs are added, this slope gradually decreases with increasing CNC content, indicating a transition from mainly viscous behavior to a more elastic and solid-like response. At high frequencies, the behavior is dominated by the polymer matrix, while at low frequencies the influence of CNC–polymer interactions becomes more important. The combined increase in G' and the reduced slope in the terminal region confirm the formation of a percolated CNC network. This effect is most evident in the DMSO-processed samples, where the weak frequency dependence of G' suggests a strongly interconnected CNC structure within the matrix.

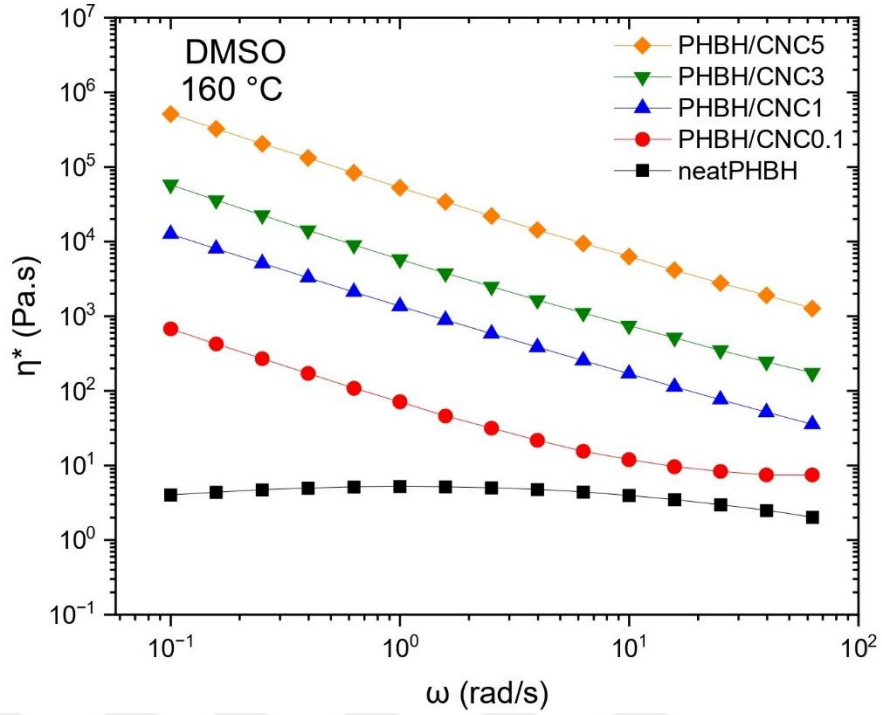

Figure 5.7 : Complex viscosity versus frequency for PHBH/CNC nanocomposites prepared in DMSO with varying CNC contents.

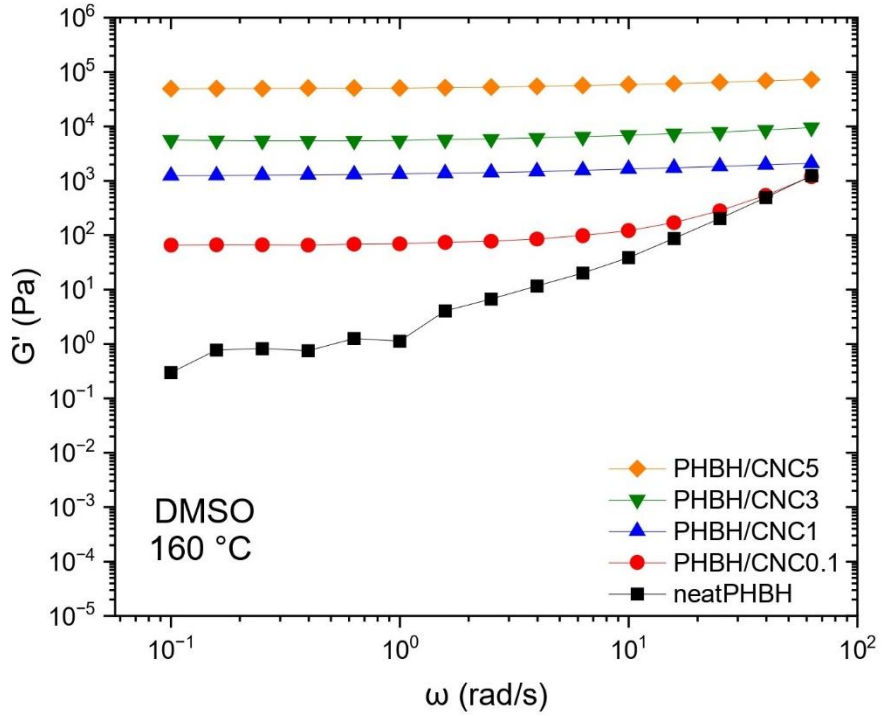

Figure 5.8 : Storage modulus versus frequency for PHBH/CNC nanocomposites produced in DMSO at varying CNC concentrations.

5.3.2 Modified Cole-Cole plot analysis

The Cole–Cole plot (G′ versus G″) is commonly used to evaluate the viscoelastic behavior and structural uniformity of polymer systems. A linear trend with a slope

close to two indicates a homogeneous polymer matrix, while any deviation suggests increased heterogeneity or network formation due to polymer–particle interactions. Figure 5.9 presents the Cole–Cole plots of neat PHBH and PHBH/CNC nanocomposites prepared with different solvents. Neat PHBH shows a nearly linear relationship, typical of polymer melts, whereas the addition of CNC causes noticeable deviations from linearity, particularly for samples prepared with DMSO. The PHBH/CNC3 DMSO nanocomposite exhibits a more pronounced curvature, indicating stronger elastic behavior and enhanced interfacial interactions between CNC and the PHBH matrix. In contrast, nanocomposites prepared with CHL and THF remain almost linear, suggesting limited CNC dispersion and weak particle–matrix adhesion. DMF-based nanocomposites show intermediate behavior, implying partial network formation. Figure 5.10 shows the effect of CNC content for DMSO-based nanocomposites. As CNC content increases, both G′ and G″ increase while the slope gradually decreases, indicating a transition from viscous to elastic response. Even at 0.1 wt.% CNC, a slight deviation from the neat PHBH line can be seen, confirming effective dispersion. At higher CNC loadings (3–5 wt.%), the strong curvature and reduced slope indicate the formation of a continuous CNC network. These result suggest that the percolation threshold for PHBH/CNC nanocomposites prepared with DMSO lies between 0–1 wt.% CNC consistent with the frequency sweep observations.

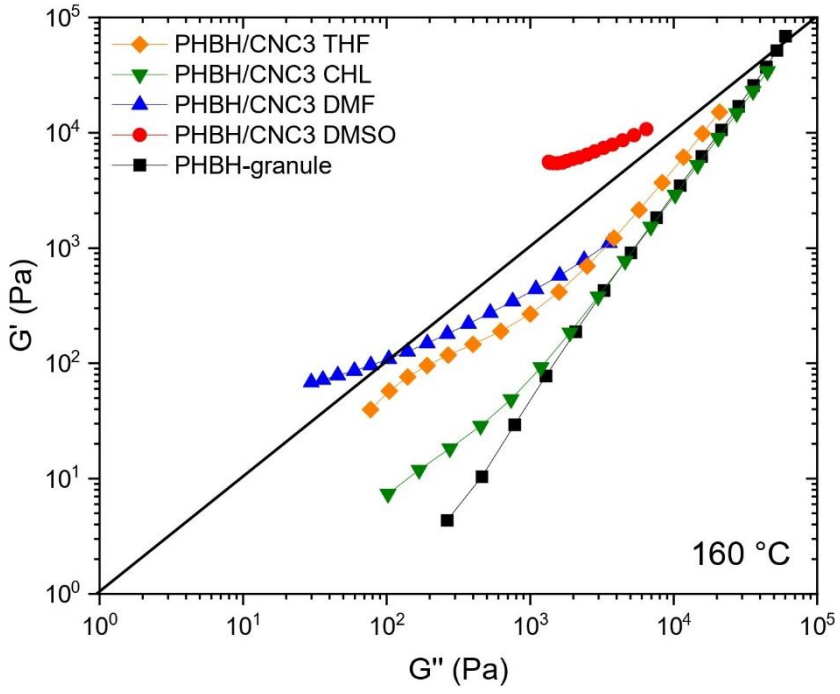

Figure 5.9 : Modified Cole–Cole plots of PHBH/CNC nanocomposites processed with DMSO, DMF, CHL, and THF.

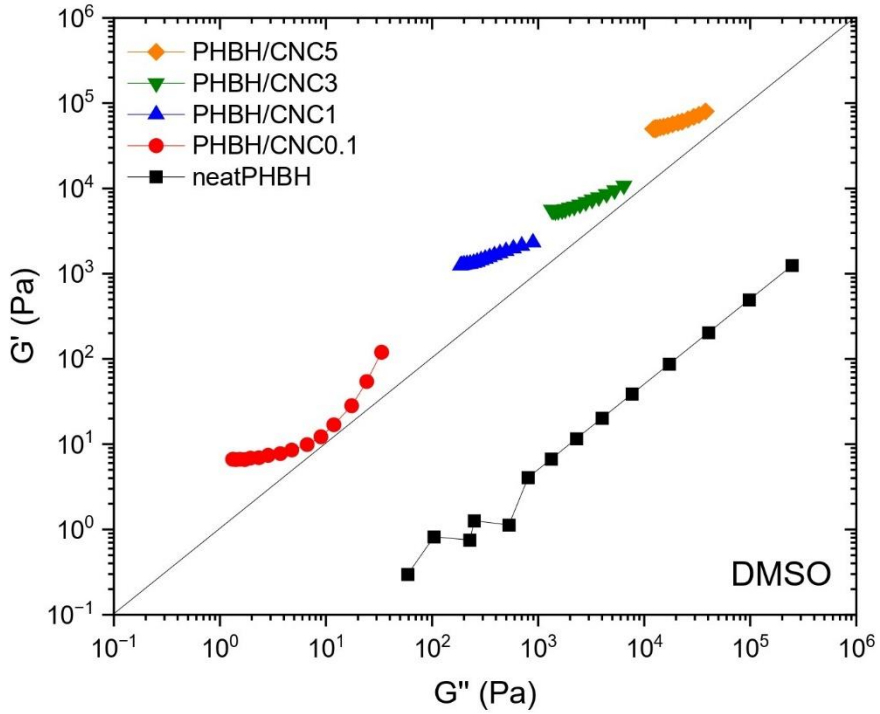

Figure 5.10 : Modified Cole–Cole representation of PHBH/CNC nanocomposites processed in DMSO.

5.3.3 Calculation of percolation threshold

The formation of a continuous CNC network was determined by applying an empirical power-law fit to the storage modulus measured at 0.1 rad s^{-1} over different CNC loadings. The power-law expression used for this fitting is given as:

$$G' = \beta\_c\, G\, ((m - m\_cG)/(m\_c\, G))\text{^}n\,, \qquad for\ m > m\_c\, G$$

where $\beta_{(cG)}$ and n are fitting constants, m refers to the CNC loading in weight percent, and $m_{(cG)}$ indicates the critical percolation concentration. Figure 5.11 presents the experimental values of the storage modulus at 0.1 rad s^{-1} plotted against the CNC content for PHBH/CNC nanocomposites produced with DMSO.

The values of mcG for NC prepared with DMSO are determined to be 0.05 weight percent. As mentioned previusly, in order to achieve beter CNC dispersion, the matrix polymer should have a lower molecular weight. For this reason, percolation threshold was calculated fort he samples prepared with the PHBH331A matrix. The result support that, even at very low concentrations, it is possible to obtain a very strong network formation by using a DMSO solution.

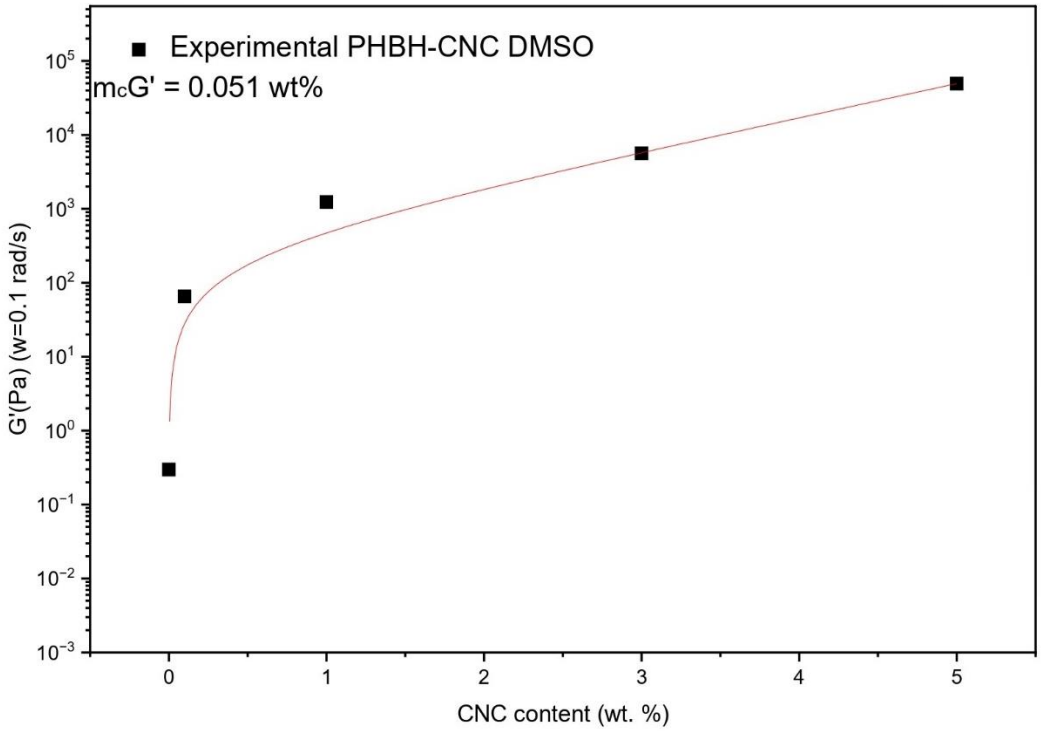

Figure 5.11 : Storage modulus at 0.1 rad s^{-1} as a function of CNC content for DMSO-processed PHBH/CNC nanocomposites, with power-law fits shown in red.

5.3.4 Apperant yield stress

Adding nanoparticles to a polymer melt can create an apparent yield stress because a filler network is formed. In PHBH/CNC nanocomposites, CNCs cause a strong increase in viscosity at low frequencies, which indicates a more solid-like viscoelastic response. This type of behavior is typical for materials that exhibit yield stress. The apparent yield stress can be evaluated from the relationship between complex viscosity and complex modulus.

Figure 5.12 illustrates the relationship between complex viscosity and complex modulus (G*) for PHBH/CNC nanocomposites. As the complex modulus decreases, a corresponding increase in complex viscosity is observed in the nanocomposites. This behaviour provides evidence the addition of CNCs leads to the formation of a percolation network structure within the matrix. Consequently, the nanocomposite acquires yield stress.

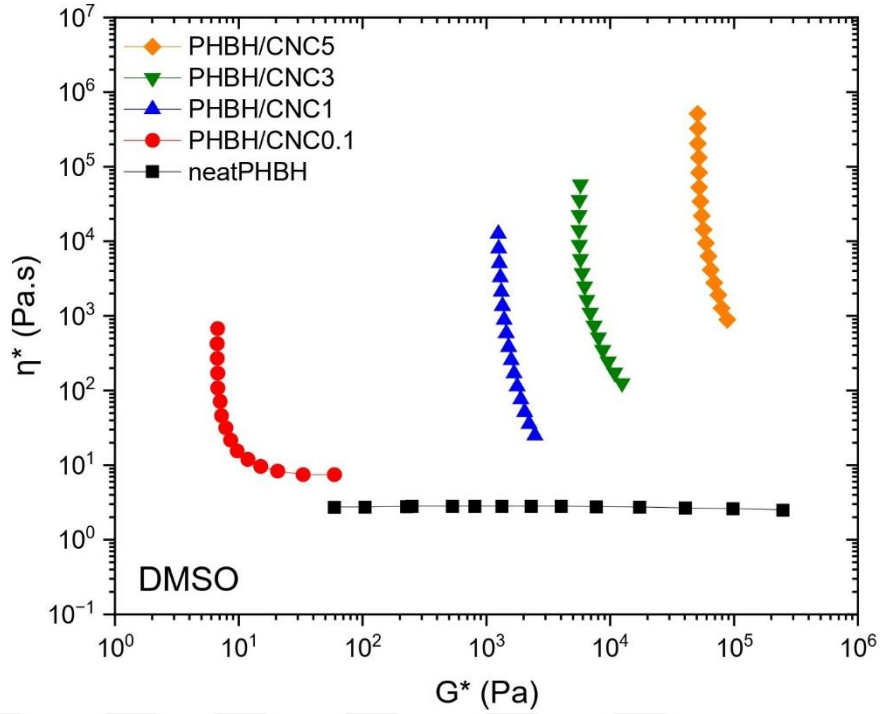

Figure 5.12 : Relationship between complex viscosity and complex modulus for PHBH/CNC nanocomposites obtained using DMSO.

Table 5.1 : Apparent yield stress and melt flow index of PHBH/CNC nanocomposites determined from the modified Herschel–Bulkley model.

	σ_0(Pa)	k(Pas^n)	n
PHBH	0	3,75	1,22
PHBH/CNC0.1	0,2	3,89	1
PHBH/CNC1	3,63	9,51	0,53
PHBH/CNC3	17,14	0	0,53
PHBH/CNC5	97,44	73,66	0,03

Additional finding further support these results. For the samples prepared using DMSO, a yield stres of 3.63 Pa was calculated even at 1wt% CNC loading. As expected, CNC incorporation also significantly altered melt flow index.

5.4 Differential Scanning Calorimetry (DSC)

Figure 5.13 presents the DSC thermograms of PHBH/CNC nanocomposites containing 1, 3, and 5 wt.% CNC prepared in DMSO. Before the measurements, all samples were heated from ambient temperature to 200 °C at a rate of 10 °C min^{-1} in order to eliminate any prior thermal history. No cold crystallisation was observed during the second heating of NCs. The DSC results clearly show that CNCs function as efficient nucleating agents within the PHBH matrix. With increasing CNC content,

the crystallization process shifts to higher temperatures during the cooling process, indicating that the presence of CNCs facilitates earlier crystal formation, which is in line with their nucleation capability.

As the CNC content increases, an increase in crystallization is generally expected. However, at high CNC loadings, CNCs may restrict the mobility of PHBH chains, which can lead to slower crystallization. Additionally, due to the high boiling temperature of DMSO, residual solvent may remain within the nanocomposite (NC) structure. These solvent residues can reduce the molecular mobility of PHBH and consequently hinder crystallization. When examining the cooling curve in Figure 5.13, the lowest crystallization observed in the 3% CNC sample can be explained in this way. Since the samples have different thermal histories, the crystallization behavior observed during the first heating does not provide a reliable basis for comparison. In the second heating curves, the 1% CNC sample exhibits a broader peak, whereas the 5% CNC sample shows a narrower peak. This indicates that in the 5% CNC nanocomposite, the crystal sizes and their distribution are more uniform and closer to each other. As the CNC content decreases, greater variations in crystal size and distribution are observed within the nanocomposite.

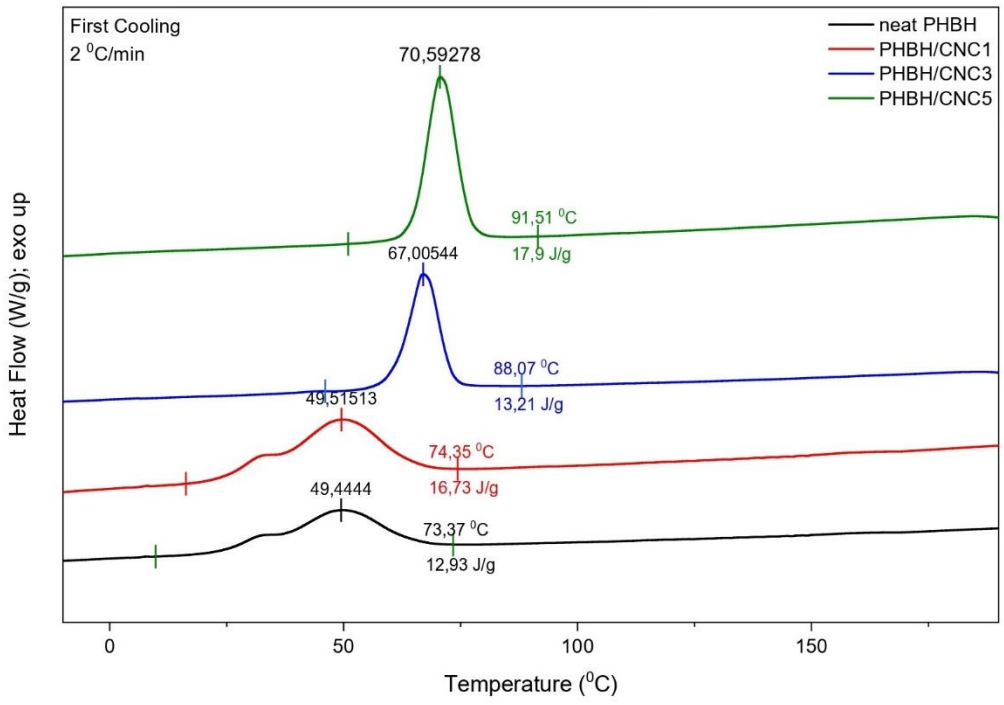

Figure 5.13 : DSC cooling–heating thermograms of PHBH/CNC nanocomposites prepared by solvent casting with DMSO (heating: 10°C/min, cooling: 2°C/min).

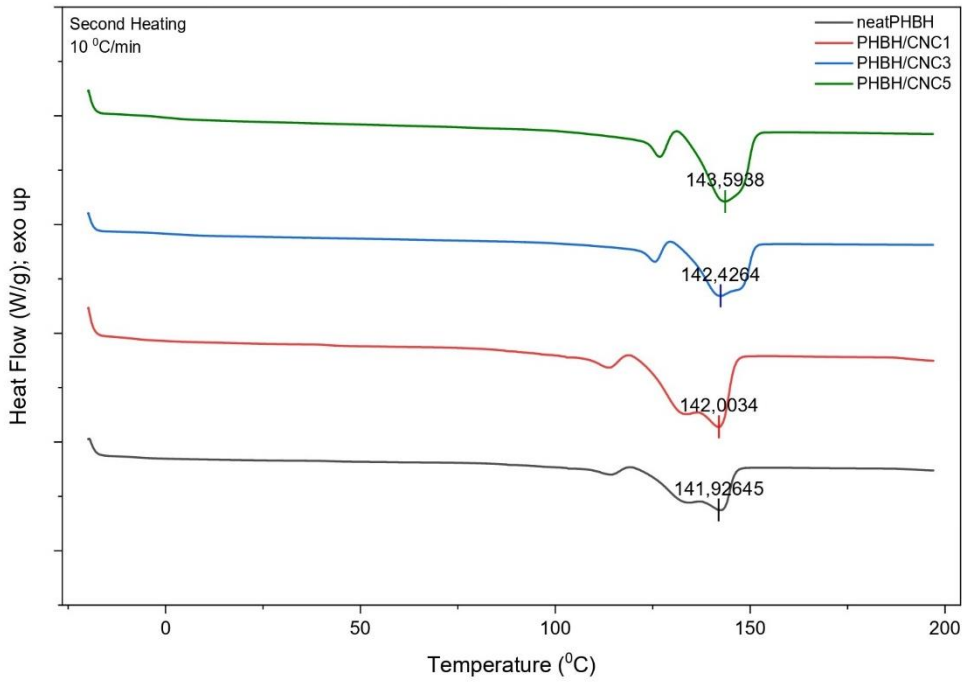

Figure 5.13 (continued) : DSC cooling–heating thermograms of PHBH/CNC nanocomposites prepared by solvent casting with DMSO (heating: 10°C/min, cooling: 2°C/min).

6. CONCLUSION

This study investigated how CNC content and solvent type affect CNC dispersion in PHBH/CNC nanocomposites, as well as their rheological and thermal properties. To examine the solvent effect, nanocomposites containing 3 wt.% CNC were prepared by the solvent casting method using DMSO, DMF, THF, and CHL.

Rheological results showed that DMSO provided the best CNC dispersion. This is mainly due to its high polarity and dielectric constant, which promote stronger interactions between PHBH and CNC. As a result, a connected CNC network formed at lower CNC contents. Among all solvents, DMSO led to the greatest improvement in rheological properties. DMF also showed some improvement, while THF and CHL had little to no effect.

For PHBH/CNC nanocomposites prepared in DMSO, the percolation threshold was determined to be 0.051wt%. Even at 0.1wt% CNC loading, a significant enhancemet in rheological properties was observed. DMF is a polar solvent and, after DMSO, has one of the highest dielectric constants. Owing to this characteristic, nanocomposites prepared in DMF also exhibited high complex viscosity values at low frequencies. In the nanocomposite containing 3 wt.% CNC prepared using DMF, although not as pronounced as in the samples prepared with DMSO, a good level of CNC dispersion was achieved.

Additionally, the addition of CNCs positively affected the crystallization behavior of PHBH by facilitating crystal formation. Therefore, crystallization started at higher temperatures during the cooling process. At higher CNC contents, the mobility of PHBH chains may decrease, which can slightly influence the crystallization efficiency. Overall, CNC incorporation improves the thermal performance of PHBH and contributes to a more homogeneous crystalline structure.

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