SYNTHESIS & CHARACTERIZATION OF NYLON–6,6 MICROPLASTICS IN DIFFERENT CONCENTRATIONS

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

SUBMITTED BY

UPASANA UPADHYAY

(2K20/MSCBIO/33)

UNDER THE SUPERVISION OF

PROF. JAI GOPAL SHARMA

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi–110042

MAY 2022

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi–110042

CANDIDATE DECLARATION

I, **Upasana Upadhyay**, **2K20/MSCBIO/33**, hereby declare that the Dissertation Project entitled **"Synthesis & Characterization of Nylon–6,6 Microplastics in Different Concentrations"**, submitted by me in partial fulfillment of the requirements for the award of the degree of Master of Science in Biotechnology, to the Department of Biotechnology, Delhi Technological University in the academic session 2021–22 is an original one. To the best of my knowledge, no part has been submitted earlier to any university for the award of any degree.

I assert the statements made and conclusions drawn are an outcome of my research work.

I further certify that

I. The work contained in the report is original and has been done by me under the general supervision of my supervisor.
II. The work has not been submitted to any other Institution for any other degree/diploma/certificate in this university or any other University of India or abroad.
III. We have followed the guidelines provided by the university in writing the report.
IV. Whenever we have used materials from other sources, we have given due credit to them in the text of the report and have given their details in the references.

Upasana Upadhyay
(2K20/MSCBIO/33)

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi–110042

CERTIFICATE

This is to certify that the dissertation project entitled **"Synthesis & Characterization of Nylon–6,6 Microplastics in Different Concentrations"**, submitted by **"Upasana Upadhyay"**, in partial fulfillment of the requirements for the award of the degree of Master of Science in Biotechnology, to the Department of Biotechnology, Delhi Technological University, Bawana Road, Delhi, is carried out by her under my supervision.

To the best of my knowledge, the matter embodied in the dissertation project has not been submitted to any other university or institute for the award of any degree or diploma.

Place: Delhi, India
Date: May 2022

Prof. Jai Gopal Sharma
Supervisor
Department of Biotechnology
Delhi Technological University

Prof. Pravir Kumar
Head of Department
Department of Biotechnology
Delhi Technological University

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Upasana Upadhyay
(2K20/MSCBIO/33)

ABSTRACT

Known to be of different shapes, colors and sizes, microplastics are defined as “any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1μm to 5 mm, of either primary or secondary origin, which are insoluble in water”. "Nylon" is one such type of plastic whose qualities make it suitable for use in a variety of applications. It is a high performance polymer with strong thermal, electrical, and chemical resistances. In this research, Nylon-6,6 beads have been used in 3 different concentrations to generate 3 different samples of microplastics. The 3 samples thus produced are then subjected to characterization to obtain the difference in the properties of the microplastics generated out of these 3 samples. This research is carried out with the objective to find out the minimum possible and most suitable particle size of microplastic with improved properties that can be acquired from any of the 3 samples, so that they can be employed in varied applications to boost the performance of the application. It is observed that sample 2 was found to contain microplastics with finest properties among the three.

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ABBREVIATIONS

MP: Microplastics

PA6: Polyamide 6

PA66: Polyamide 66

PA 6-3-T: Polytrimethylene Hexamethylene Terephthalamide

TGA: Thermogravimetric analysis

FT-IR: Fourier Transform InfraRed

TEM: Transmission Electron Microscopy

µm: Micrometer

PdI: Polydispersity Index

GLOSSARY

Abrasion Resistance: An ability to withstand the wear and tear of friction caused by mechanical parts or instances of repetitive scraping or rubbing.

Cam bearings: A Camshaft with one or more cams attached to it, especially one operating the valves in an internal combustion engine. Cam bearings support the camshaft and allow it to rotate.

Dimensional Stability: The degree to which a material maintains its original dimensions when subjected to changes in temperature and humidity.

Exfoliating product: A product which removes dead skin cells from the surface of your skin using a granular substance called an exfoliant.

Fishing line: A long thread of silk or nylon attached to a baited hook, with a sinker or float, and used for catching fish.

Impact Strength: It is the amount of energy that a material can withstand when the said load is suddenly applied to it.

Landfills: It is a site for the disposal of waste materials.

Pyrolysis: The pyrolysis process is the thermal decomposition of materials at elevated temperatures in an inert atmosphere.

Tensile Strength: Resistance to lengthwise stress, measured by the greatest load in weight per unit area pulling in the direction of length that a given substance can bear without tearing apart.

Thermal Expansion: The general increase in the volume of a material as its temperature is increased.

Virgin plastic pellets: Unused or never processed or pure (new) plastic pellets.

Yield Stress: The minimum stress at which a material will deform without significant increase in load.

CHAPTER 1: INTRODUCTION

Known to be of different shapes, colors and sizes, "microplastics are defined as any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1μm to 5 mm, of either primary or secondary origin, which are insoluble in water" (Seghers et al., 2022). "NOAA Marine Debris Program of the US defined microplastics as plastic particles smaller than 5.0 mm in size" (Masura et al., 2015).

Based on their origin, Microplastics can simply be categorized into two main classes: primary microplastics and secondary microplastics. Primary microplastics are polymers that are purposefully designed to be microscopic in size. (Cole et al., 2011). These include purposefully produced raw plastic materials such as virgin plastic pellets and microbeads. An extensive usage of primary microplastics has been reportedly seen in the cosmetic industry, where such microplastics in the form of microbeads are incorporated as exfoliating agents in the exfoliating products. Until 1980, elements such as pumice, oatmeal and ground almonds are being used as exfoliants in exfoliating products. However since 1980, microplastics in the form of microbeads are being used in exfoliating products as exfoliating agents (Cverenkárová et al., 2021). Moreover, their use in medicine as vectors for drugs has also been reported. They are also been seen to be used as potential air blasting media as well (Cole et al., 2011). Whereas, primary microplastics are originally designed to be tiny in size, secondary microplastics arise from the breakdown of bigger plastic products. Secondary microplastics are produced by the unintentional disintegration of big plastic products such as plastic bags, cartons, ropes, and nets. These huge bits of plastic degrade into smaller and smaller plastic particles over time (Cverenkárová et al., 2021) as they undergo mechanical, biological and/or photo (oxidative) degradation (Masura et al., 2015).

These secondary sources of microplastics are considered as the main source of microplastics in the environment (Seghers et al., 2022).

With the beginning of the industrial production of plastics in 1950, approx. 2 million tonnes of plastics were being generated annually since then. However, by 2015, plastic output had increased to over 380 million tonnes per year worldwide. More than 7800 million tonnes of plastic were created between 1950 and 2015, resulting in 6300 million tonnes of garbage, out of which around 9% was recycled. (Cverenkárová et al., 2021) Only 12% of this garbage was burned, with the remaining 79% ending up in landfills or the environment, where it may take centuries for such debris to break down and degrade. (Cole et al., 2011). Much of this plastic waste ends up in the aquatic environment (Cverenkárová et al., 2021). It enters in the aquatic environment both directly as well as indirectly such as from landfills, from where plastics enter into the rivers, seas and oceans via wind. Plastics are predicted to reach the seas and oceans in the amounts of 8 million tonnes per year (Cverenkárová et al., 2021).

In recent years, environmental contamination with microplastics (MP) has gotten a lot of attention from the general public. MP is increasingly becoming a significant worldwide concern, mostly due to the breakdown of bigger plastic products (Ritchie & Roser, 2020). Microplastic particles have been discovered in all compartments of the aquatic and terrestrial habitats, where they can aggregate and persist for lengthy periods of time. There is also growing worry that MP contamination of water, food, soil, and air poses a threat to the environment and human health (Raamsdonk et al., 2020). Microplastics have been detected in a variety of creatures, including vertebrates and invertebrates. Scientists are also concerned that creatures swallowing plastic trash might be exposed to toxins that have been attached to the plastic (Teuten et al. 2007). Plastic waste serves as both a sink and a source of chemical pollutants. Additives used in the manufacture of plastics can seep into the marine environment (Andrady 2011). Hydrophobic pollutants in the water, on the other hand, may bind to the plastic particles (Carpenter et al. 1972). As a result, microplastics may serve as a vehicle for the delivery of concentrated pollutants to organisms (Browne et al. 2007). Thus, microplastics have lately become a hot topic among scientists (Cverenkárová et al., 2021).

Despite knowing the fact that plastics pose a potential risk to the environment, they are still continuously being manufactured across the globe due to the exceptional properties they possess.

"Nylon" is one such type of plastic whose qualities make it suitable for use in a variety of applications. Nylons are basically included in the class of polyamides. Polyamides (nylon) are high performance polymers with strong thermal, electrical, and chemical resistances. They are “widely utilized in automotive and transportation industry, consumer products, and electrical and electronics applications, among others” (Cverenkárová et al., 2021). In this backdrop, the present study was carried out using Nylon-6,6 where Nylon-6,6 beads have been used in 3 different concentrations to generate 3 different samples of microplastics. This research is carried out with the objective to find out the minimum possible and most suitable particle size of microplastic with improved properties that can be acquired from any of the 3 samples, so that they can be employed in varied applications to boost the performance of the application.

CHAPTER 2: REVIEW OF LITERATURE

2.1. Nylon: Chemistry, Types, Properties and Uses

2.1.1. Nylon: Introduction

Nylon is a polymer that is widely used. It is a component of several apps that are critical in our daily lives. As a result, it is critical to research the chemistry and properties of nylon, which are crucial for its use in a variety of applications (Vagholkar, 2016). Electrical connectors, cam bearings, gear, slide, cable ties and film packaging, fishing line, fluid reservoirs, brush bristles, fabric, sports and recreational equipments are some of the common applications of these nylons. (Vagholkar, 2016).

2.1.2. Nylon: Chemistry and Types

"Nylons are basically included in the class of polyamides" (Vagholkar, 2016). Being a polymer that is found everywhere around us, Nylon is a component of several applications that play critical role in our day to day lives. As a result, it is in turn critical to investigate the chemistry and characteristics of nylon, which are vital in determining its suitability for diverse uses (Vagholkar, 2016). Nylons are polyamides i.e. long chain synthetic polymeric amides since they possess recurring amide groups as an integral part of the main polymer chain (Mufaddal Bagwala, 2013). They are high performance polymers with excellent thermal, electrical, and chemical resistances.

Nylons can be formed via 2 ways:

A. By condensation polymerisation between dicarboxylic acids and diamines as shown in Figure 1. Nylons made from condensation of a dicarboxylic acid and a diamine are classed based on "the number of carbon atoms present in the amine and acid respectively." (Mufaddal Bagwala, 2013) For example, nylon synthesized by condensation polymerization of "hexamethylene diamine (NH_2 $(CH_2)_6$-NH_2) that

contains 6 carbon atoms and sebacic acid (COOH-$(CH_2)_8$-COOH) that contains 10 carbon atoms is normally denoted as nylon 6,10" (Mufaddal Bagwala, 2013).

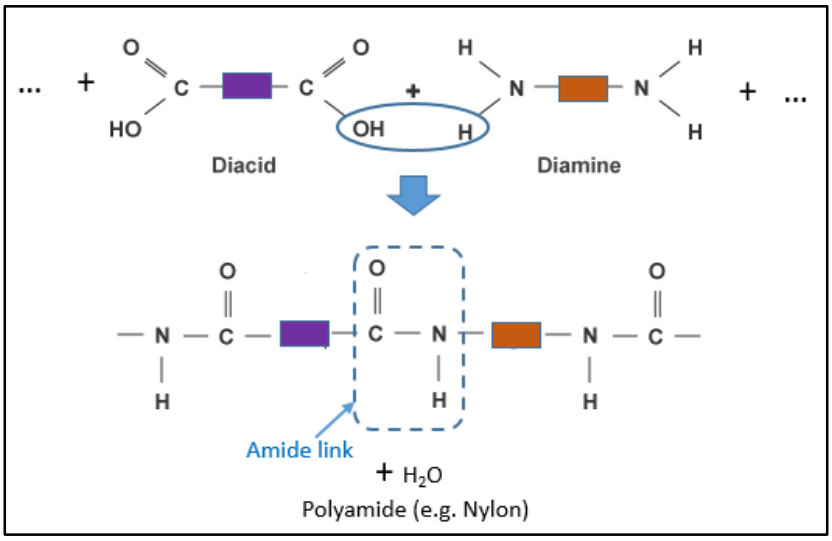


Figure 1: Condensation polymerization reaction of Nylons.

B. Nylons made solely from amino acid. These are classed based on the number of carbon atoms present in the acid. For example, Nylon 6 is synthesized from polymerizing an amino acid that contains 6 carbon atoms i.e., either from amino caproic acid or from its condensed product caprolactum as shown in figure 2 (Mufaddal Bagwala, 2013).

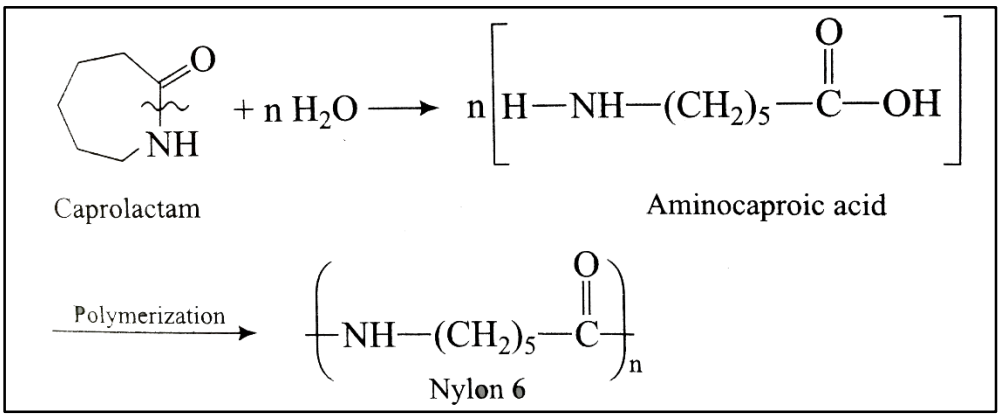


Figure 2: Nylon-6 formed by ring opening polymerization of ε-Caprolactam

So, polyamides (or Nylon) are often synthesized by either polycondensing a "diacid with a diamine or by ring-opening polymerization of lactams with 6, 11 or 12 carbon atoms. The monomers may be aliphatic, semi-aromatic or aromatic" (Omnexus: The material selection platform, n.d.). They can be amorphous, semi-crystalline or can have varing degrees of crystallinity (Omnexus: The material selection platform, n.d.).

Table 1: Aliphatic polyamides and their monomers

Polyamide (Nylon)	Monomer(s)
1. Nylon 4,6	1. 1, 4 diamino butane, Adipic acid
2. Nylon 6,6	2. Hexamethylene diamine, Adipic acid
3. Nylon 6, 1 0	3. Hexamethylene diamine, Sebacic acid
4. Nylon 6,12	4. Hexamethylenediamine, Dodecanedioic acid
5. Nylon 3	5. Acrylamide
6. Nylon 4	6. 2-pyrolidane
7. Nylon 6	7. Caprolactum
8. Nylon 7	8. Lactum of heptonoic acid
9. Nylon 11	9. W-amino-cendecanoic acid
10. Nylon 12	10. Dodelactum

Aromatic polyamides (also known as Aramids) are created by polycondensing terephthalic acid with diamines. PA 6-3-T is a popular example of an aromatic polyamide that is amorphous and transparent in nature. These materials may be treated at temperatures ranging from 280 to 300°C. Morever, when compared to aliphatic polyamides, aramids are found to be more costly, but they are found to offer a greater dimensional stability, flame and heat resistance, and strength. (Omnexus: The material selection platform, n.d.)

2.1.3. Nylon-6 vs Nylon-6,6

"Polyamide 6 (PA6), also known as, Nylon-6 or polycaprolactam is one among the most widely used polyamides on a global scale" (Omnexus: The material selection platform, n.d.). It is produced by ring-opening polymerization of caprolactam (Refer figure 3). PA6 has a melting point of 223°C (Omnexus: The material selection platform, n.d.).

"While, Polyamide 66 (PA66) or Nylon-6,6 is one of the most often used engineering thermoplastics. It is primarily employed as a metal substitute in a variety of applications" (Omnexus: The material selection platform, n.d.). Nylon-6,6 is made by polycondensing two monomers containing six carbon atoms each i.e. hexamethylenediamine and adipic acid (Refer figure 3). Polyamide 66 has a melting point of 255°C.

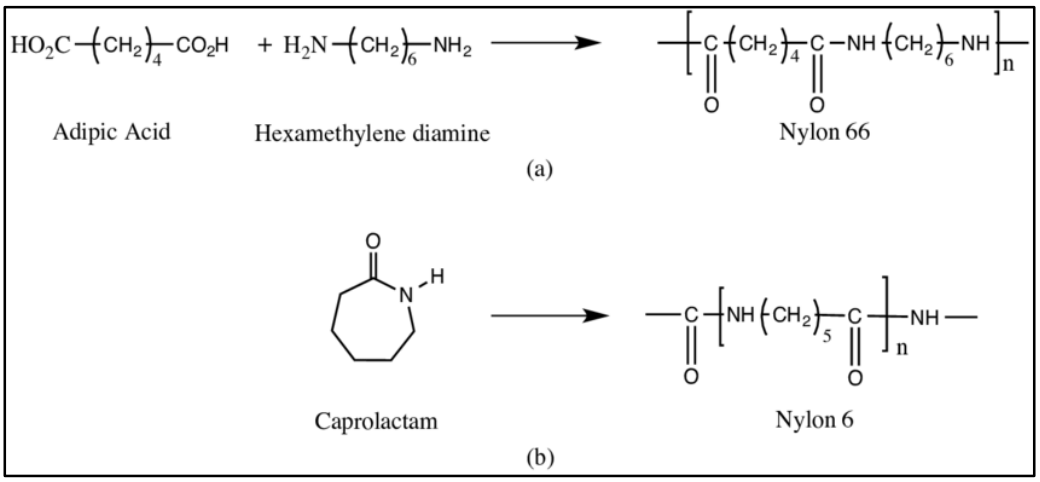


Figure 3: Polymerization reaction of – (a) nylon-6,6 and (b) nylon-6

PA6 and PA66 are by far the most widely utilized polyamides on a worldwide scale. Because of their high performance/cost ratios, both polyamides 6 (PA6) and 66 (PA66) are widely employed in a variety of markets and applications. The following are some of their most important characteristics (Omnexus: The material selection platform, n.d.).

- High strength and stiffness at high temperature
- Good impact strength, even at low temperature
- Very good flow for easy processing
- Good abrasion and wear resistance
- Excellent fuel and oil resistance
- Good fatigue resistance
- PA 6 has excellent surface appearance and better processability than PA66 (due to its very low viscosity)
- Good electrical insulating properties
- High water absorption and water equilibrium content limits the usage
- Low dimensional stability
- Attacked by strong mineral acids and absorbs polar solvents
- Proper drying before processing is needed

Howsoever, there are various differences between them, despite the fact that they share many similar characteristics. PA6 has a lower temperature resistance yet is much less costly than PA66.

In comparison to PA6, the PA66 has: Nylon 66 outperforms nylon 6—the other big volume nylon—in many applications, thanks to its improved dimensional stability, higher melting point, stronger tensile strength, excellent abrasion resistance and more compact molecular structure (Mufaddal Bagwala, 2013).

Nylon-6, 6 has a higher strength than Nylon-6. This is simply due the increased levels of hydrogen bonding in case of Nylon-6,6 (Omnexus: The material selection platform, n.d.) (Refer figure 4). Because of this increase in the levels of hydrogen bonding between the neighboring chains, the melting temperature and crystallinity also increases. As crystallinity rises, so do the following things: "Stiffness, Density, Tensile and yield stress, Chemical and abrasion resistance and better dimensional properties" (Vagholkar, 2016).

Increased crystallinity, on the other hand, reduces: Elongation, Impact resistance, Thermal expansion and Permeability (Vagholkar, 2016).

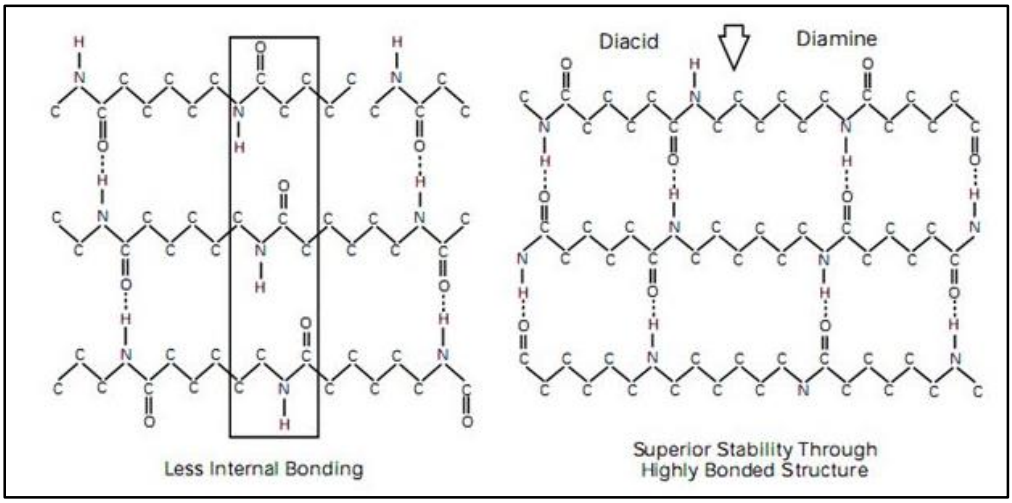


Figure 4: The molecular structure of nylon-6 vs nylon-6,6

2.2. Overview of different characterization techniques employed:

2.2.1. Thermogravimetric analysis (TGA)

TGA is a valuable method for assessing the thermal stability of materials such as polymers. In this process, changes in the weight of a specimen are measured while its temperature is raised. It can also determine the volatile elements and moisture content of a sample as well. So, "Thermogravimetric analysis (TGA) instruments can measure a host of parameters like moisture loss, decarboxylation, pyrolysis, loss of solvent, loss of plasticizer, oxidation, and decomposition for biomass or other substances" (Ebnesajjad & Ebnesajjad, 2006).

2.2.2. Zeta Potential Analysis

The zeta potential is a physical property that any particle in suspension exhibits (Malvern Instruments Limited, 2015). Zeta potential is, basically, the measurement of the effective electric charge present on the particle's surface. The zeta potential is caused by the net electrical charge contained within the region bounded by the slipping plane (Refer figure 5).

The magnitude of the zeta potential indicates particle stability, with particles with higher magnitude zeta potentials demonstrating more stability due to greater electrostatic repulsion between particles (nanoComposix, n.d.). Low zeta potential colloids tend to coagulate or flocculate.

Size and zeta potential are unrelated – a bigger particle just has more surface charge, hence the zeta potential remains constant regardless of size.

PDI, in zeta, "is essentially a representation of the distribution of size populations within a given sample. The numerical value of PDI varies from 0.0 (for a perfectly uniform sample with respect to the particle size) to 1.0 (for a highly polydisperse sample with multiple particle size populations)" (Danaei et al., 2018).

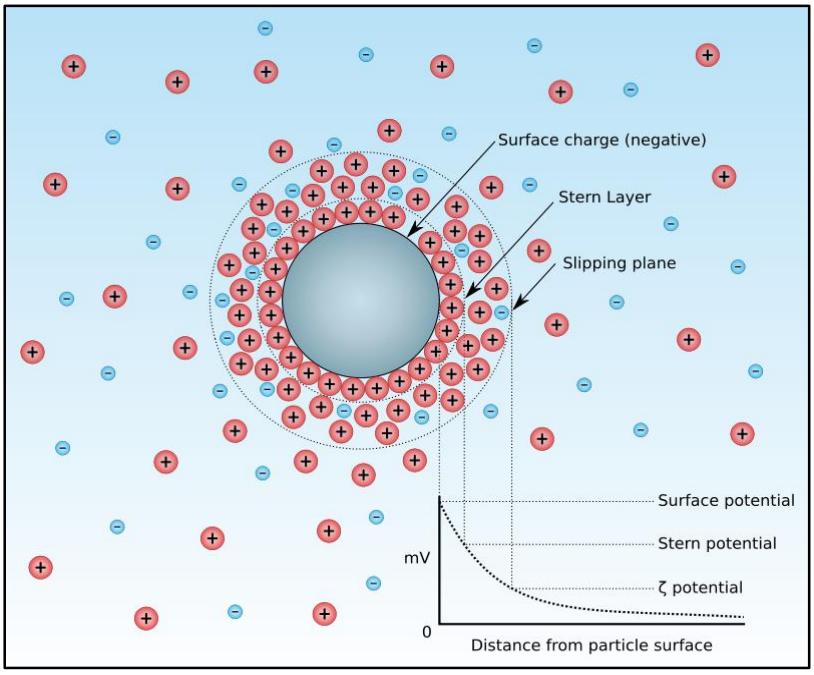


Figure 5: Diagram displaying the ionic concentration and potential difference of a particle suspended in a dispersion media as a function of distance from the charged surface.

2.2.3. Fourier Transform-InfraRed (FT-IR)

Fourier transform infrared (FTIR) is the preferred method of infrared spectroscopy. When infrared radiation is sent through a sample, part of it is absorbed and some flows through. The resultant signal at the detector is a spectrum that represents the sample's molecular "fingerprint." The use of infrared spectroscopy stems from the fact that various chemical structures (molecules) yield different spectral fingerprints. So, the Fourier Transform turns the detector output to an interpretable spectrum and produces spectra with informative patterns that provides useful structural insights about the chemical molecule (ThermoFisher Scientific, n.d.).

2.2.4. Transmission electron microscope (TEM)

Transmission electron microscopy (TEM) allows for the resolution of features as small as 1 A°. A beam of electrons is sent through a thin sample in TEM, where the electrons

are either absorbed, scattered or are transmitted through the specimen (Pednekar et al., 2017).

The image is generated by focusing the transmitted electrons on a fluorescent screen. The picture contrast is caused by variations in mass-thickness, with thicker portions of the specimen absorbing or scattering more electrons than thinner sections (Pednekar et al., 2017).

CHAPTER 3: MATERIALS AND METHODS

3.1. Materials Required:

3.1.1. For sample preparation:

I. Chemicals used:

a) Nylon-6,6 beads

b) Formic Acid, Pure, 85%

II. Equipment used:

a) Magnetic stirrer and Magnetic beads

b) Weighing balance

III. Glasswares/Other items used:

a) Beakers

b) Graduated cylinder

c) Aluminum Foil

3.1.2. For Sonication of the Sample:

I. Chemicals used:

a) MilliQ water

b) Sodium Hydroxide pellets

c) Acetone

II. Equipment used:

a) Ultra-Sonicator

III. Glasswares/Other items used:

a) Beakers

b) Ice

c) Tissue paper

3.1.3. For Centrifugation of the sonicated sample:

I. Chemicals used:

a) MilliQ water

II. Equipment used:

a) Bench Top Cooling Centrifuge

III. Glasswares/Other items used:

a) Beakers

b) Centrifuge tubes/Falcon Tubes

c) pH strips

3.1.4. For drying of the centrifuged sample:

I. Equipment used:

a) Hot Air Oven

3.1.5. For Characterization of the samples:

I. Equipment used:

a) Zeta Potential Analyzer

b) Thermogravimetric Analyzers (TGA)

c) A Fourier Transform InfraRed (FT-IR) Spectrometer

d) Transmission Electron Microscope (TEM)

3.2. Methodology:

3.2.1. Sample Preparation:

- Nylon-6,6 beads were taken to prepare 3 different samples having different concentration of nylon in each. The samples were prepared first by measuring the amount of Nylon-6,6 using weighing balance and then dissolving it in a suitable solvent i.e. formic acid.
- The three different samples having different concentrations of nylon were prepared as follows:

Table 2: Three different samples having different concentrations of nylon

	Nylon-6,6 beads (in grams)	**Formic Acid (in ml)**
Sample 1	1g	50ml
Sample 2	2g	50ml
Sample 3	3g	50ml

- Then, three beakers were taken (for sample 1, sample 2 and sample 3) and 50-50ml of formic acid was added to each. Graduated cylinder was used to measure 50ml formic acid each time.
- To the three beakers, the nylon-6,6 beads were then added as per the amounts mentioned in the above table.

- Magnetic beads were then added to the beakers after which the beakers were covered using aluminum foil. The samples were then mixed thoroughly using magnetic stirrer.
- On complete dissolution of the nylon beads, the 3 samples were then taken for sonication.

3.2.2. Sonication:

- One by one, the 3 samples were then subjected to ultra-sonication.
- The sonication set up was such that 2 beakers were taken one inside the other (small beaker inside the larger one) and ice was filled in between the two. The ice looks after the overheating issues of the device.

Figure 6: Ultra Sonicator Set Up

- The small beaker was then filled with MilliQ water.

- The first sample was taken, a small aliquot was poured into the small beaker containing the MilliQ water and this was then subjected to sonication for 5 min. After 5 min, again a small aliquot of the sample was poured into the beaker and subjected to sonication for another 5 min. This process was repeated until the sample got over. But, whenever it was noticed that sample started to form fiber like structures inside the small beaker, the water in the beaker was changed i.e. it was collected into a separate beaker and fresh water was added to the small beaker inside the sonicator. The process was then resumed till the sample got over.
- Other two samples were also subjected to sonication the same way. It was made sure that before using the sonicator for a new sample, the sonicator probe was neutralized with a base like sodium hydroxide (as the sample contained acid in it) and cleaned using solvents like acetone.
- After the sonication process was completed, the bulk water that was collected into separate beakers during the sonication of each sample solution (3 beakers obtained for 3 sample solutions) was then taken for centrifugation.

3.2.3. Centrifugation:

- One by one, the 3 beakers were then subjected to centrifugation.
- For each sample beaker, six 50ml falcon tubes were taken.
- The centrifugation process was then started with the first sample. 6 falcon tubes reserved for the first sample were then filled with equal volumes of the sample so as to maintain the balance of the centrifuge. The lid of the centrifuge was then opened, proper rotor that could hold the 50ml falcon tubes was chosen, tubes were placed inside the rotor maintaining the balance, and the lid was then tightly secured again. Finally, the desired settings were selected i.e. the rotor speed, the temperature and the time for which one want to run the rotor. In this case, the centrifuge was set at 12000rpm for 8min at 25ºC (RT). After centrifugation, the tubes were removed from the centrifuge and the supernatant was discarded while not disturbing the pellet obtained. The same tubes were

again filled with the same sample and subjected to centrifugation again. This process continues till the sample got over.

- The other two samples were also subjected to centrifugation the same way. Finally, 6 tubes per sample were obtained from this step, each containing a pellet.
- Then, to remove any acidic remnants from the pellets, the pellets were washed at least 3 times with milliQ water using a centrifuge. The acidic content for each pellet was then checked using pH strips. If the acidic content persisted, the pellets were then again washed for 2-3 times.

3.2.4. Drying:

- The 3 sets of six tubes were then dried in a hot air oven for few hours with their lids open.
- After drying, for each set, dried pellets from their respective 6 tubes were then transferred to one.
- At the end of this step, 3 tubes containing dried pellets of 3 different concentrations of nylon-6,6 were obtained. These dried pellets are further crushed to obtain a powdered form of each sample.
- These dried pellets/powder obtained at the end was nothing but microplastics made from different concentrations of nylon-6,6.
- These 3 samples were then further given for characterization of the microplastics obtained in each case.

3.2.5. Characterization:

- In this, first the samples were subjected to Zeta potential analysis so as to identify that one sample that contains microplastics possessing finest properties. On identification of that sample, to know more about the sample, the sample was further characterize using different characterization techniques which are as follows:

a) Thermogravimetric Analysis (TGA)

b) A Fourier Transform InfraRed (FT-IR) Spectroscopy

c) Transmission Electron Microscopy (TEM)

CHAPTER 4: RESULTS

4.1. Zeta Potential Analysis

4.1.1. Size Distribution Report

A) Sample 1

Size Distribution Report by Intensity
v2.2

Malvern

Sample Details

Sample Name:	Sample 1 1
SOP Name:	mansettings.nano
General Notes:	

File Name:	Sachin.dts	**Dispersant Name:**	Water
Record Number:	955	**Dispersant RI:**	1.330
Material RI:	1.52	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	25 March 2022 10:53:16

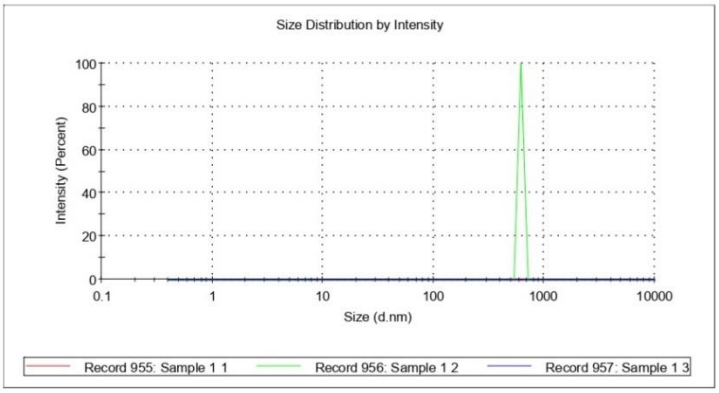
System

Temperature (°C):	25.0	**Duration Used (s):**	120
Count Rate (kcps):	91.3	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	6

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	3.038e4	**Peak 1:**	0.000	0.0	0.000
PdI:	0.691	**Peak 2:**	0.000	0.0	0.000
Intercept:	2.04	**Peak 3:**	0.000	0.0	0.000

Result quality Refer to quality report



Malvern Panalytical
www.malvernpanalytical.com

Zetasizer Ver. 7.13
Serial Number : MAL1220421

File name: Sachin
Record Number: 955
25 Mar 2022 11:02:04

B) Sample 2

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name: sample 2 3
SOP Name: mansettings.nano
General Notes:

File Name:	Sachin.dts	**Dispersant Name:**	Water
Record Number:	963	**Dispersant RI:**	1.330
Material RI:	1.52	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	25 March 2022 11:16:03

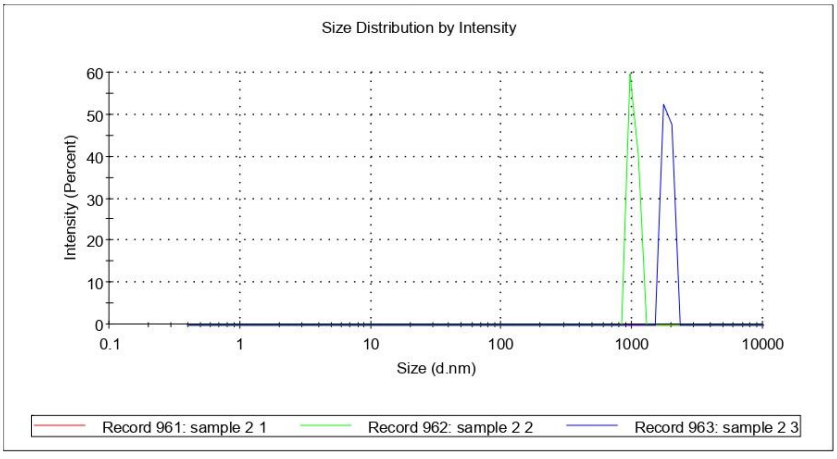
System

Temperature (°C):	25.0	**Duration Used (s):**	60
Count Rate (kcps):	419.0	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	8

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	7257	**Peak 1:**	1848	100.0	135.7
PdI:	1.000	**Peak 2:**	0.000	0.0	0.000
Intercept:	1.01	**Peak 3:**	0.000	0.0	0.000

Result quality Refer to quality report



C) Sample 3

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name: sample 3 3
SOP Name: mansettings.nano
General Notes:

File Name:	Sachin.dts	**Dispersant Name:**	Water
Record Number:	969	**Dispersant RI:**	1.330
Material RI:	1.52	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	25 March 2022 11:31:46

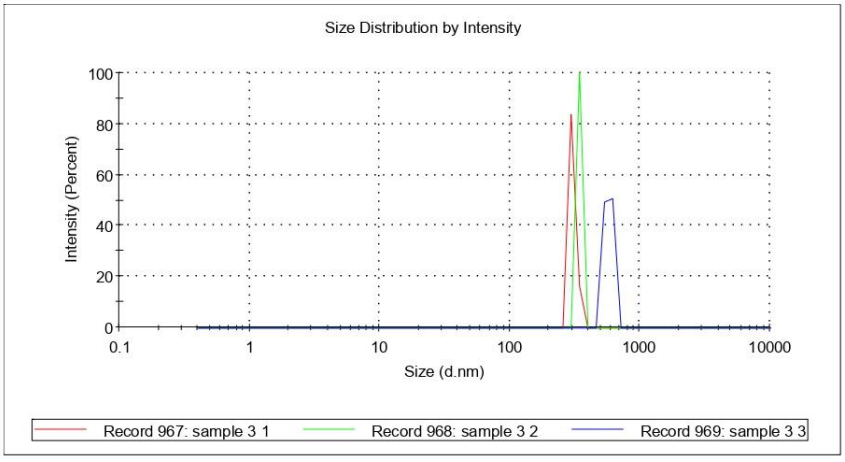
System

Temperature (°C):	25.0	**Duration Used (s):**	70
Count Rate (kcps):	120.8	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	9

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	5145	**Peak 1:**	573.7	100.0	41.98
PdI:	0.547	**Peak 2:**	0.000	0.0	0.000
Intercept:	1.08	**Peak 3:**	0.000	0.0	0.000

Result quality Refer to quality report



4.1.2. Zeta Potential Report

A) Sample 1

Zeta Potential Report

v2.3

Malvern

Sample Details

Sample Name: sample 1 3
SOP Name: mansettings.nano
General Notes:

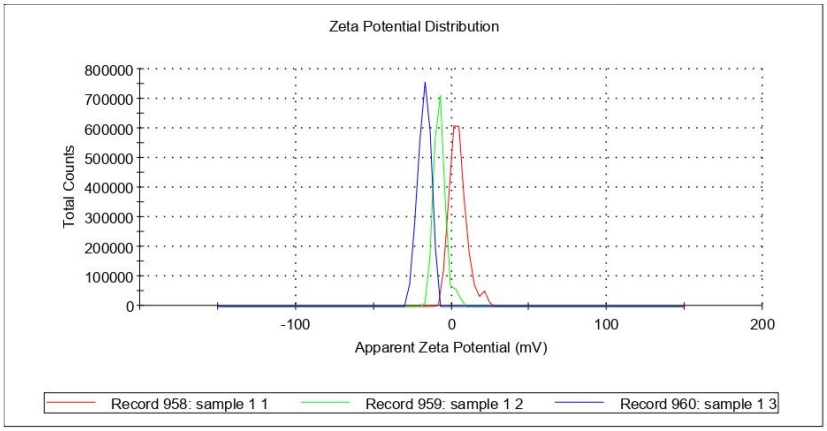
File Name:	Sachin.dts	**Dispersant Name:**	Water
Record Number:	960	**Dispersant RI:**	1.330
Date and Time:	25 March 2022 11:06:30	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	**Zeta Runs:**	12
Count Rate (kcps):	2432.7	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta c...	**Attenuator:**	11

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-23.8	**Peak 1:**	-17.4	100.0	4.05
Zeta Deviation (mV):	36.5	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.0810	**Peak 3:**	0.00	0.0	0.00
Result quality	Good				



B) Sample 2

Zeta Potential Report

v2.3

Malvern

Sample Details

Sample Name: sample 2 3

SOP Name: mansettings.nano

General Notes:

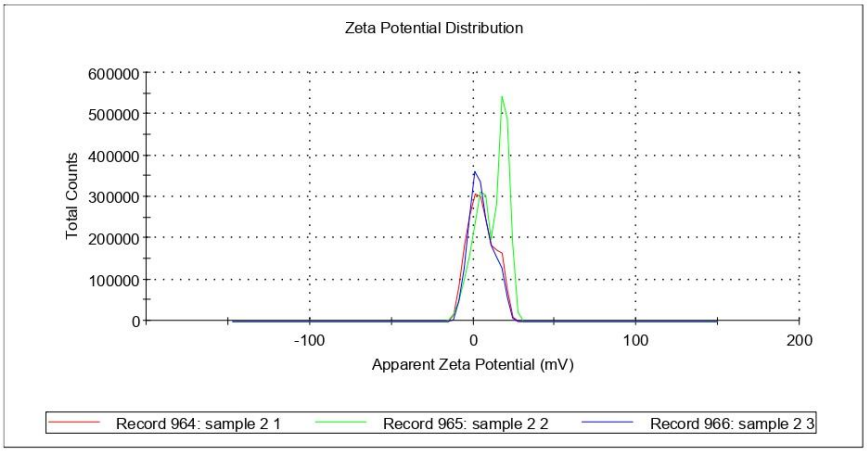
File Name:	Sachin.dts	**Dispersant Name:**	Water
Record Number:	966	**Dispersant RI:**	1.330
Date and Time:	25 March 2022 11:20:56	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	**Zeta Runs:**	12
Count Rate (kcps):	1645.3	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta c...	**Attenuator:**	9

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	5.19	**Peak 1:**	5.19	100.0	7.34
Zeta Deviation (mV):	7.34	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.889	**Peak 3:**	0.00	0.0	0.00
Result quality	Good				



C) Sample 3

Zeta Potential Report

v2.3

Malvern

Sample Details

Sample Name: sample 3 3

SOP Name: mansettings.nano

General Notes:

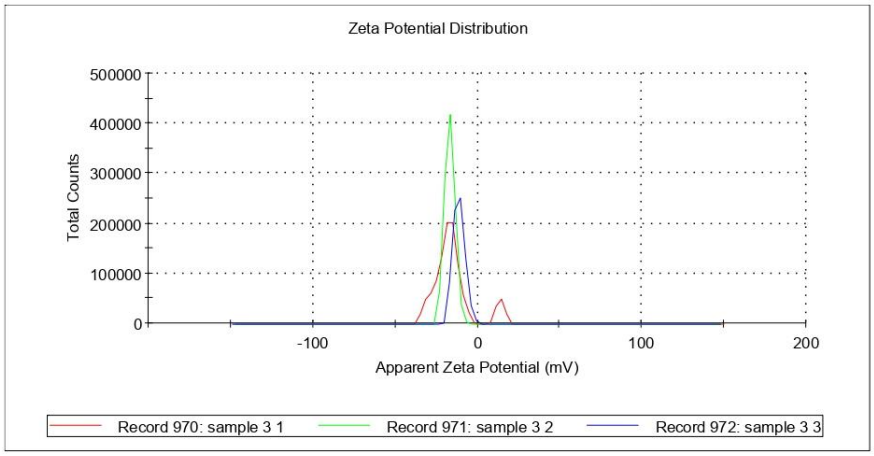
File Name:	Sachin.dts	**Dispersant Name:**	Water
Record Number:	972	**Dispersant RI:**	1.330
Date and Time:	25 March 2022 11:38:06	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	**Zeta Runs:**	12
Count Rate (kcps):	77.1	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta c...	**Attenuator:**	9

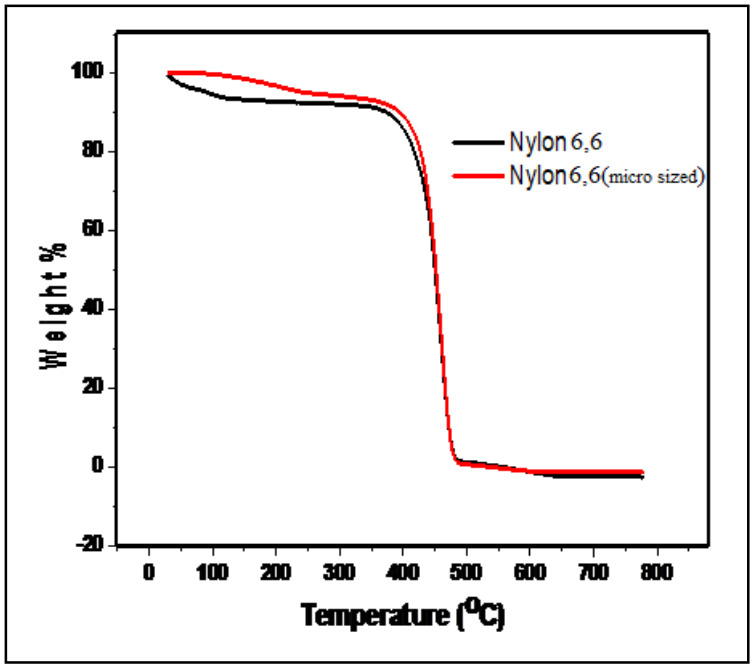
Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-11.2	**Peak 1:**	-11.2	100.0	3.59
Zeta Deviation (mV):	3.59	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.133	**Peak 3:**	0.00	0.0	0.00
Result quality	Good				



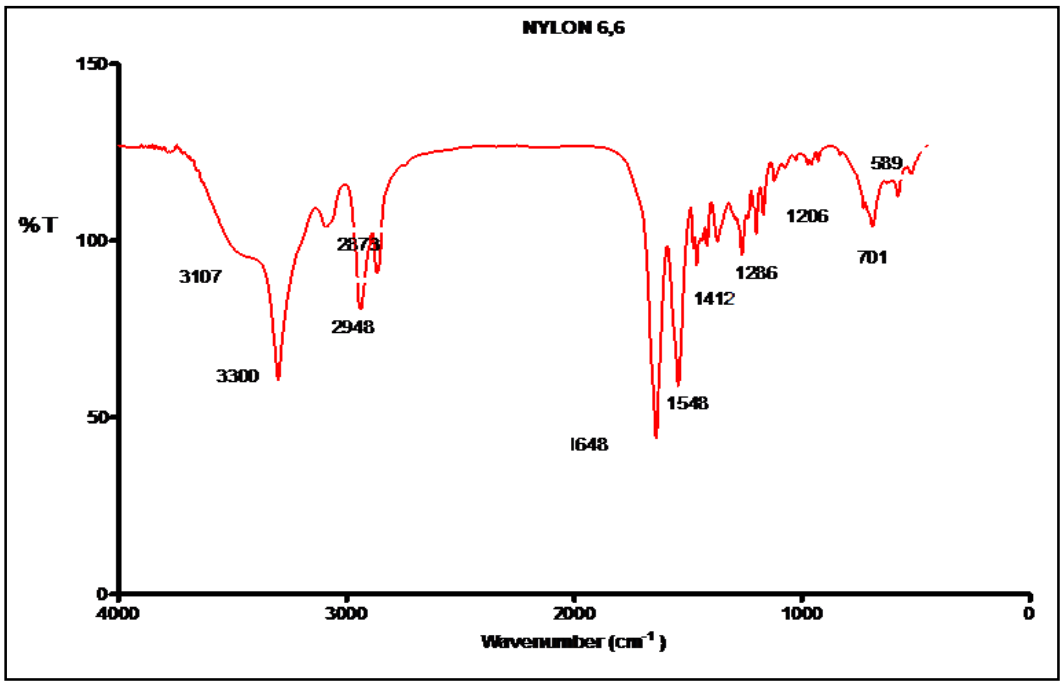
4.2. Thermogravimetric Analysis (TGA)

A) Sample 2



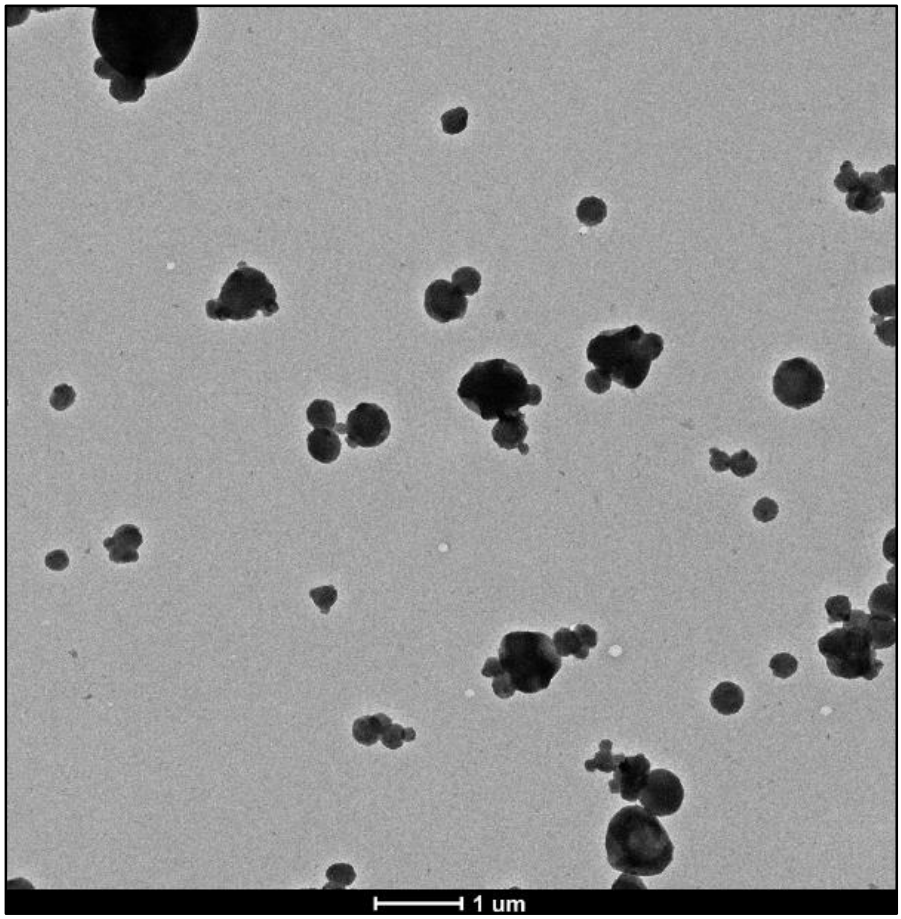
4.3. Fourier Transform InfraRed (FT-IR) Spectroscopy

A) Sample 2



4.4. Transmission Electron Microscopy (TEM)

A) Sample 2



CHAPTER 5: DISCUSSION AND CONCLUSION

Table 3: Data obtained from zeta potential analysis

	Sample 1	Sample 2	Sample 3
Size (d.nm)	**3.038×10^4**	**7257**	**5145**
Polydispersity Index (PdI)	**0.691**	**1.000**	**0.547**
Zeta Potential (mV)	**-23.8**	**5.19**	**-11.2**

Based on the data obtained from Zeta potential analysis of the 3 samples, sample 2 was found to contain microplastics with finest properties among the three. This can be said as it has a zeta potential value of 5.19 which is the highest among the three samples. The more the zeta potential, more will be the charge and hence more will be the particle stability. However, it has a Polydispersity Index highest among the three. This means that sample 2 is a highly polydisperse sample containing multiple particle size populations. Hence, it also has the highest average particle size among the three samples.

Then, as sample 2 was found to contain microplastics having finest properties, it was sent for further characterization. First, TGA was performed. TGA of nylon-6,6 microplastics showed slight increase in the thermal stability with respect to the reference material used i.e. nylon-6,6 beads. Then, FTIR was performed to know the sample's molecular fingerprint. The FTIR of nylon-6,6 microplastics showed the presence of amide groups which is well reflected as a strong band at about 1648 cm^{-1} and an intense band at 3300 cm^{-1}. Finally, a TEM image is generated for the sample 2 microplastics which showed nylon-6,6 particles with approximate size of 200-400nm.

Conclusively, the minimum possible and most suitable particle size of microplastic with improved properties was found to be the microplastics obtained from sample 2.

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APPENDIX

Appendix A. List of Publications

- **Upadhyay, U**., Kamal, P., Sharma, J.G. (2022). *Impact of Oral versus Injectable Antibiotics in the Development of Antibiotic Resistance.* Proceedings of ACN International Conference (pp. 67-69). Institute for Technology and Research (ITRESEARCH).

INTERNATIONAL CONFERENCE ON ENVIRONMENT, AGRICULTURE AND BIOTECHNOLOGY (ICEABT)

Dear Researcher,

Many Congratulations to you!!!!

We are happy to inform you that your paper entitled **"Impact of oral versus injectable antibiotics in the development of Antibiotic resistance"** has been selected for ACN- International Conference on Environment, Agriculture and Biotechnology (ICEABT) on 28th Apr 2022 at Delhi, India.

IMPORTANT INFORMATION:

Paper Title	Impact of oral versus injectable antibiotics in the development of Antibiotic resistance
Universal paper ID(Note for future communication)	ACN-EABT-DELH-280422-201
Conference Website	http://academicsconference.com/Conference/21471/ICEABT/
Last Date of Registration	23RD Apr, 2022

NOTE:Your paper has also cleared theStage-1(out of two stages)for publication in the upcoming issues of any one of the following International Journals after 30 to 45 Days of the Event.

- International Journal of Electrical, Electronics and Data Communication(IJEEDC),12 Issues/Year
  Journals Impact Factor(JIF)-3.46Indexing- DRJI, BASE Indexing, Google Scholar, OAJI, Jour Informatics
- International Journal of Mechanical and Production Engineering(IJMPE),12 Issues/Year
  Journals Impact Factor(JIF)-3.05Indexing- DRJI, BASE Indexing, Google Scholar, DOAJ, OAJI
- International Journal of Advance Computational Engineering and Networking(IJACEN),12 Issues/Year
  Journals Impact Factor(JIF)-3.2, SJIF-3.89 Indexing- DRJI, BASE Indexing, Google Scholar, OAJI
- International Journal of Soft Computing And Artificial Intelligence(IJSCAI),2 Issues/Year
  Journals Impact Factor(JIF)-1.95Indexing- DRJI, Google Scholar
- International Journal of Advances in Computer Science and Cloud Computing(IJACSCC), 2 Issues/Year
  Journals Impact Factor(JIF)-2.05Indexing- DRJI, , Google Scholar
- International Journal of Advances in Science, Engineering and Technology(IJASEAT) ,4 Issues/Year
  Journals Impact Factor(JIF)-3.15Indexing- DRJI, Google Scholar, OAJI
- International Journal of Industrial Electronics and Electrical Engineering(IJIEEE), 12 Issue/Year
  Journals ImpactFactor(JIF)-3.2Indexing- DRJI, Google Scholar, OAJI
- International Journal of Advances in Mechanical and Civil Engineering (IJAMCE),12 Issue/Year
  Journals Impact Factor(JIF)-3.64Indexing- Google Scholar
- International Journal of Advances in Electronics and Computer Science (IJAECS),12 Issue/Year
  Journals Impact Factor(JIF)-2.68Indexing- Google Scholar
- International Journal of Management and Applied Science (IJMAS),12 Issue/Year
  Journals Impact Factor(JIF)-3.98Indexing- Google Scholar

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ACADEMICS CONFERENCE NETWORK

International Conference on

Environment, Agriculture and Biotechnology

Venue: New Delhi, India | Date: 28th April, 2022

CERTIFICATE

OF PRESENTATION

This is to certify that

Upasana Upadhyay

for presenting a paper entitled "Impact of Oral Versus Injectable Antibiotics in The Development of Antibiotic Resistance" at the International Conference on Environment, Agriculture and Biotechnology (ICEABT) held in New Delhi, India on 28th April, 2022.

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IMPACT OF ORAL VERSUS INJECTABLE ANTIBIOTICS IN THE DEVELOPMENT OF ANTIBIOTIC RESISTANCE

[1]JAI GOPAL SHARMA, [2]UPASANAUPADHYAY, [3]PRAGYA KAMAL

Delhi Technological University (DTU), Delhi -110 042, India
E-mail: [1]sharmajaigopal@dce.ac.in

Abstract - Antibiotics have revolutionized the whole vision towards infectious diseases. They are considered as "miracle drugs" since they were discovered in 1940s. It can be said that the whole terror of bacterial disease that use to cause havoc in human lives is no longer to be feared. Since their discovery, antibiotics have been used widely all over the world to treat multiple diseases. Antibiotics were boon until the concept of antibiotics came into picture where the dosage and the administration are the two things that are needed to be considered while using them as both of these factors can contribute to antibiotic resistance. Dosage is something which is consider while prescribing and taking antibiotics but still negligence can been seen when it comes to administration of antibiotics. This present review sheds light on this issue that route of administration of antibiotics plays an role in enhancing the antibiotic resistance.

Keywords - Antibiotics, Oral antibiotics, IV (Intravenous), Antibiotic resistance.

I. INTRODUCTION

Antibiotics are substances that have antibacterial properties and are used to treat bacteria borne infections or diseases. There are two kinds of antibiotics: a) Bacteriocidal that kills the bacteria by interfering with its life dependent mechanisms like preventing the formation of cell wall and b) bacteriostatic that inhibit the bacterial growth by preventing its reproduction.(Nankervis et al., 2016) Antibiotics do not work against viruses, however, there are few antibiotics that can work against certain protozoan for example Metronidazole has both antibacterial and antiprotozoal activity.(American Society of Health-System Pharmacists (ASHP), 2021) Antibiotics are antibacterialsi.e. they work against bacteria, but these two terms antibiotic and antibacterial are used in very different senses although they seem almost the same.

According to the medical vocabulary, substances which are only naturally derived and used in medication are known as antibiotics like penicillin and substances that are synthetically made are called "nonantibioticantibacterials" or simply antibacterials.Examples of this category are antiseptics and disinfectants which are chemically derived and are not suitable to be used as medicines for humans or animals. However, the basic function of either antibiotic or antibacterial products is to stop bacterial growth or kill the bacteria, but usage of these terms refined during the course of time and are now used to refer two different things. With the introduction of antibiotics a dramatic reduction took place towards infectious diseases,yet new bacterial diseases keep on coming into sight.This, together with the increased usage of existing antibiotics cause the antibiotic resistance which still remains an area of major concern.

II. ANTIBIOTIC ADMINISTRATION AND ANTIBIOTIC RESISTANCE

From soil to skin, food and hence feces, large populations of antibiotic resistant bacteria have been found prevailing the varied ecosystems across the globe. Bacterial populations may possess antibiotic resistance even against the antibiotics that they do not even produce. Oral exposure of such antibiotic resistance bacteria serve as the main channel via which resistant strains are disseminated to humans in their GI tract.(Zhang et al., 2013)

On administration of antibiotics, due to the amplification of pre-existing antibiotic resistant bacterial population and emergence of endogenous resistant strains, the overall resistant bacterial population increases in the gut.(Zhang et al., 2013)This is simply due to the fact that resistant bacteria transfer resistant genes across the bacterial population residing. Through sequencing experiments, it was revealed that generally, β-lactamases and a large number of homologous genes have been found to be transferred most commonly. Resistant genes often form clusters as they are transferred together. (Fair & Tor, 2014)Moreover, resistant bacteria have also been found to alter or utilize their existing metabolic machinery to source the resistant determinants. An example of this phenomenon could be the transfer of efflux pumps and immunity genes from the resistant bacteria to other bacteria. Once transferred, efflux pumps and immunity genes become an exclusive source of resistance determinants.(Zhang et al., 2013)

Once emerged, because of the niche fitness, the antibiotic resistant bacteria persist in the gut of the host. Hence, it can be rightly said that dissemination, amplification, emergence and persistence of antibiotic resistant bacteria in the gut of the host, together,

contributes to the prevalence of antibiotic resistance in humans.(Zhang et al., 2013)

III. IMPACT OF ANTIBIOTIC ADMINISTRATION ROUTES ON ANTIBIOTIC RESISTANCE

As oral exposure serve as the main gateway for antibiotic resistance bacteria to be disseminated to humans, GI tract/gut is primarily considered for determining the impact of antibiotic administration routes on antibiotic resistance. There are two modes via which antibiotics are administered i.e. oral mode of administration and administration via intravenous (IV) mode. But, the rise in antibiotic resistance in the gut directly depends on the type of antibiotic being administered as different antibiotics have different drug excretion routes. For example, in case of ampicillin, ampicillin is excreted mainly via renal routes. So, when ampicillin is administered via IV mode, there is less chance that pre-existing antibiotic resistant bacterial population in the gut will get access to the antibiotic. However, in case of, for example tetracycline, it is excreted both via renal pathways (glomerular filtration) and GI tract (direct elimination and biliary elimination). So, even if tetracycline is injected intravenously, the pre-existing antibiotic resistant bacterial population will somehow get access to the antibiotic. Nevertheless, the oral administration of any antibiotic whatsoever will result in the increase of antibiotic resistance in the gut of the host as pre-existing antibiotic resistant bacterial population in the gut will get the direct access to the antibiotic. So, conclusively it can be said that, the rise in antibiotic resistance in the gut is especially prominent when antibiotics are introduced orally. Moreover, on comparison of ampicillin and tetracycline, the difference in antibiotic resistance between oral and IV administration routes is much more significantly seen for ampicillin than for tetracycline.(Zhang et al., 2013)

On the other hand, when considering infections at locations in the body other than GI tract such as in the bones and spine, intravenous mode of antibiotic administration is preferred “as intravenous antibiotics penetrate tissues quicker and at higher concentrations than oral antibiotics”(Johnson, 2017). However, for majority of cases, oral route of administration is preferred over the intravenous route due to varied advantages such as lower drug cost, absence of cannula-related infections and no need for a health professional or equipment to administer antibiotics. Even if in cases where IV application is needed, switch over to oral antibiotics is recommended as benefits of IV is limited to first few days of treatment only. Furthermore, for cases such as those of infections in the bones and joints where prolonged IV application is recommended, research is going on for determining the possibility of switching over to oral antibiotics too.Despite knowing that switch over to oral antibiotics is necessary,patients in hospitals are often given antibiotics via IV mode. Prolonged use of intravenous antibiotics can also, therefore, result in the increase of antibiotic resistance. (McCarthy & Avent, 2020)

IV. SOME MOST PROBLEMATIC ANTIBIOTIC RESISTANT BACTERIA

Many dangerous gram positive and gram negative bacteria are emerging as potential threats to humans as they are developing alarming antibiotic resistances to some of the most crucial antibiotics and classes of antibiotics. To name some are methicillin resistant Staphylococcus aureus (MRSA), vancomycin resistant Staphylococcus aureus(VRSA), vancomycin resistant Enterococci(VRE), penicillin resistant Streptococcus pneumoniae, multi-drug resistant (MDR) Mycobacteriumtuberculosis, multi-drug resistant (MDR) Clostridium difficile, multi-drug resistant (MDR) Pseudomonas aeruginosaand multi-drug resistant (MDR) Acinetobacter.(Fair & Tor, 2014)

V. ANTIBIOTIC USE AND ACCELERATION OF THE EVOLUTION OF BACTERIAL RESISTANCE

Antibiotic use has accelerated the evolution of bacterial resistance in many ways(Fair & Tor, 2014):

a. Over prescription of antibiotics by doctors.
b. Over use of antibiotics by general public due to lack of knowledge. People take antibiotics for infections that may not be even caused by bacteria. According to a European survey carried out in 2009, majority of the people were found to have taken antibiotics for influenza, a viral infection, unaware of the fact that antibiotics do not kill viruses.
c. Overly long antibiotic treatment regimens.
d. Over use of antibiotics in animal feed stocks. An example of this can be the over use of glycopeptide antibiotic Avoparcin as a growth inducer in food animals which led to the emergence of VancomycinResistant Enterococci (VRE) in Europe.
e. Over use of antibiotics on fruits. Antibiotics such as Oxytetracycline and aminoglycoside antibiotic Streptomycin are being sprayed on fruits like apples and pears in huge amounts.
f. Waste run-off containing antibiotics or antibiotic resistant bacteria from large agro-business plants may serve as a potential meansof facilitating the disseminationof resistance elements in varied ecosystems.

Some important strategies employed to curb the overuse of antibiotics and thus accelerating rise in

antibiotic resistances are(Fair & Tor, 2014)(McCarthy & Avent, 2020):

a. Antibiotic stewardship programs
b. Reductions in antibiotic usage
c. Cycling usage between antibiotic classes
d. d)Use of combination therapies
e. Avoiding the use of broad spectrum and last resort antibiotics whenever possible

VI. CONCLUSION

Since their discovery, antibiotics are continuously helping us overcoming many infectious bacterial diseases. Despite knowing the fact that overuse of antibiotics are paving the way for accelerating rise in antibiotic resistances, the use of antibiotics cannot be restricted, else the human and animal health will be at stake. In this regard, mainly the "food animalindustryisconcernedthatlimitingtheuseofantibiotics in food animal production will compromise the disease prevention and productivity"(Zhang et al., 2013).Though, the custom use of antibiotics for treatment purposes cannot be stopped, antibiotic overuse can be controlled so as to curb the increase of antibiotic resistance.

VII. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

VIII. FUNDING

There is no funding to report.

IX. CONFLICTS OF INTEREST

The authors report that there are no conflicts of interest in this work.

X. ETHICAL APPROVALS

Not applicable.

XI. DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

XII. ACKNOWLEDGEMENT

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I, **Upasana Upadhyay**, **2K20/MSCBIO/33**, hereby declare that the Dissertation Project entitled **"Synthesis & Characterization of Nylon–6,6 Microplastics in Different Concentrations"**, submitted by me in partial fulfillment of the requirements for the award of the degree of Master of Science in Biotechnology, to the Department of Biotechnology, Delhi Technological University in the academic session 2021–22 is an original one. To the best of my knowledge, no part has been submitted earlier to any university for the award of any degree.

I assert the statements made and conclusions drawn are an outcome of my research work.

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I. The work contained in the report is original and has been done by me under the general supervision of my supervisor.

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IV. Whenever we have used materials from other sources, we have given due credit to them in the text of the report and have given their details in the references.

Upasana Upadhyay

Upasana Upadhyay
(2K20/MSCBIO/33)

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi–110042

CERTIFICATE

This is to certify that the dissertation project entitled **"Synthesis & Characterization of Nylon–6,6 Microplastics in Different Concentrations"**, submitted by **"Upasana Upadhyay"**, in partial fulfillment of the requirements for the award of the degree of Master of Science in Biotechnology, to the Department of Biotechnology, Delhi Technological University, Bawana Road, Delhi, is carried out by her under my supervision.

To the best of my knowledge, the matter embodied in the dissertation project has not been submitted to any other university or institute for the award of any degree or diploma.

Place: Delhi, India
Date: May 2022

Prof. Jai Gopal Sharma
Supervisor
Department of Biotechnology
Delhi Technological University

06/05/2022

Prof. Pravir Kumar
Head of Department
Department of Biotechnology
Delhi Technological University

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Upasana Upadhyay.

Upasana Upadhyay
(2K20/MSCBIO/33)