Title	未利用芳香族アミノ酸 4-アミノ桂皮酸を用いた芳香族バイオベースポリマーの開発
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Development of aromatic bio-based polymers derived from unused aromatic amino acid, 4-aminophenylalanine.

By

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for the degree of
Doctor of Philosophy

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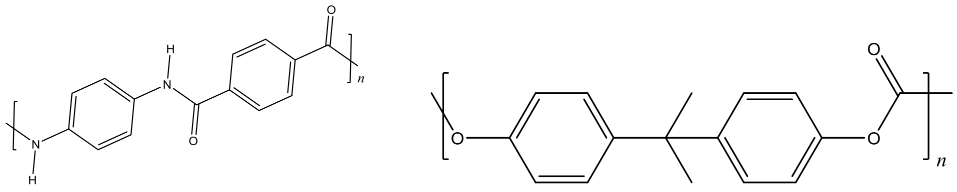
Chapter 1

General Introduction

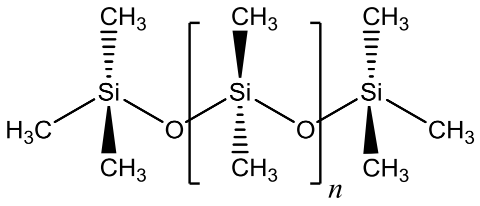
Chapter 1

1.1.*Plastic revolution-contribution, impact and concern*

After the accidental discovery of vulcanized rubber (1-4) by American Scientist Charles Goodyear in the mid-1800s, the development of synthetic polymers (5-10) gained momentum. The enormous benefits of polymers such as Bakelite (11-16) (discovered by Leo Baekeland in 1907), Neoprene (17) (discovered by Chemist and Catholic priest Julius A. Nieuwland and Arnold Collins, a chemist at the Dupont Company in the lab of Wallace Carothers in early 1900s), Nylon (18-20) (discovered in the early 1930s, by Wallace Carothers and his team of chemists at Dupont), poly(vinyl chloride) (21, 22) (discovered by German chemist Eugen Baumann in 1872), Polystyrene (23, 24) (discovered by German apothecary Eduard Simon in 1839), and others helped shape the future of polymers.



Kevlar^{TM} Lexan^{TM}



Polydimethylsiloxane

Figure 1: Polymers used during 2nd world war.

World War II further added to the rapid discoveries and pressure to bring more materials. Wartime demands and shortages pressurized scientists and researchers to develop substitutes or new materials to combat the existing materials. Spurred by the needs in the fields of electronics, medical, telecommunications, food, marine, aerospace, transporation, and other industries, a large number of materials were developed based on material performance. It is rightly said that necessity is the mother of invention. For instance, the aromatic nylons, Kevlar™ (25, 26) (capable of stopping a speeding bullet and used as tire cord) and Nomex (26) (used in fire resistant garments), polycarbonates such as Merlon™ and Lexon™ which substituted glass in various automotive products, polytetrafluoroethylene, a slick material also known as Teflon (27, 28); polysiloxanes (29), also known as silicones, which have an extremely wide temperature range tolerant, and were used as component of soles of shoes used by astronauts that first landed on moon, polyester fibres and other plastics (30, 31)such as poly(ethylene terephthalate), abbreviated as PET which is widely used in carbonated drink bottles or storing beverages.

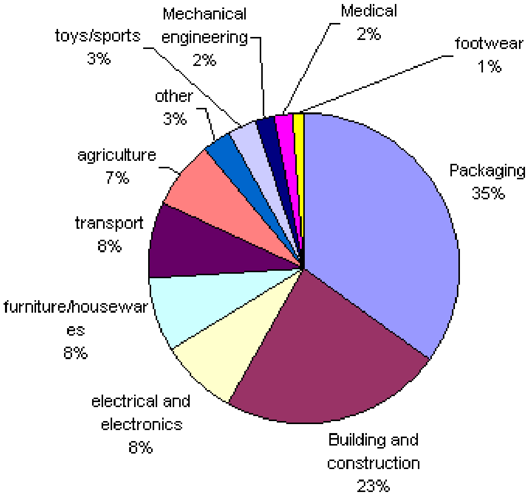


Figure 2: Contribution of plastics in various fields. Source: Kohei Watanabe, "Waste and Sustainable Consumption" March 2005; Association of Regional Planners and Architects, Detailed Sorting and Measuring of Household Waste, Kyoto 1998.

Today, polymers play an essential and ubiquitous role in everyday life. Human beings have become so dependent on them that polymer industry is the backbone of present era. They are found commonly in a variety of consumer products such as money, super glue, furniture, transportation materials, aircrafts, space-crafts, etc. The various factors which contribute to the popularity of plastics are relative low cost, versatile nature, ease of manufacturing and processability, moisture impervious nature, chemical and thermal resistant, and easy availability of raw materials. As a result, they are available in broad range of products from paper clips to spaceships. The further dominance of plastics can also be judged from the fact that they have replaced most of the traditional materials such as paper, leather, wood, ceramic, horn and bone, and are having high aesthetic values over these traditional materials. In developed countries like Japan, one third of the plastic is used in packaging whereas other main uses are occupied in building materials such as piping used in plumbing materials, furniture, automobiles, toys, etc. In the developing countries, for example India, 42% of platics is used as packaging material because of the greater demand and customers whereas in other areas, the ratios may be varied. In the medical field also, plastics occupy a large portion with products ranges from syringes, gloves to large equipment and tools. The following section covers some of the commonly used plastics (32-40) and their uses.

1. Polyester (PES): Mainly as textiles materials or fibers.
2. Polyethylene terephthalate (PET): Commonly labelled as PET bottles used for storing carbonated drinks or other liquors. Also used in microwave packaging and packing.
3. Polyethylene (PE): also called as polythene, used as supermarket bags and plastic bottles.
4. High-density polyethylene (HDPE): Used in detergent or soap bottles, milk and juice jugs, and also in molded plastic cases.
5. Polyvinyl chloride (PVC): Known for their usage in plumbing pipes and guttering, shower curtains, window frames.
6. Polyvinylidene chloride (PVDC): used in food packaging, widely known as Saran (wrap).

7. Low-density polyethylene (LDPE): Shower curtains, floor tiles etc.
8. Polypropylene (PP): Bottle caps, drinking straws, food containers, appliances, pipe systems.
9. Polystyrene (PS): Mainly known for their usage in disposable cups, cutlery, compact disk, cassette boxes, and plastic table wares.
10. High impact polystyrene (HIPS): used in refrigerator liners, food packaging, vending cups.
11. Polyamides (PA or Nylons): Mainly used as Fibers, toothbrush bristles, fishing lines, low-strength machine parts such as engine parts or gun frames.
12. Acrylonitrile butadiene styrene (ABS): Famous for their usage in electronic cases in computers, monitors, printers, keyboards, and drainage pipes.
13. Polyethylene/ Acrylonotrile Butadiene Styrene (PE/ABS): The slippery blend of PE and ABS has been commonly used in low-duty dry bearings, which are strong and tough.
14. Polycarbonate (PC): Used in eyeglasses, compact discs, security windows, traffic lights. Iphone 5C body is mainly polycarbonate.
15. Polycarbonate/Acrylonitrile Butadiene Styrene (PC/ABS): A resin blend of PC and ABS is used for creating a stronger plastic mainly used in car exterior and interior parts and also mobile phone bodies.
16. Polyurethanes (PU): Commonly used plastic in cars in cushioning foams, thermal insulation foams, surface coatings, printing rollers etc.

High performance or special purpose plastics (41-52):

1. Maleimide/ bismaleimide: Mainly used in high temperature composite materials.
2. Melamine formaldehyde (MF): It is one of main amino based plastics called as aminoplast, used in unbreakable crockery, toys for children, decorated top surface layer of the paper laminates (e.g. Formica).
3. Plastarch Material: They are mainly used in heat resistant material, mainly composed of genetically modified corn starch.

4. Phenolics or phenol formaldehydes: Bakelite, Formica, Oasis etc. are world-wide renowned phenolics used for a wide range of products. Excellent features include high modulus, relatively heat resistant and excellent fire resistant. Accordingly they are used as insulating parts in electrical fixtures, paper laminated products e.g. Formica. Bakelite is used in heat resistant products such as transistors, radio and telephone casings, firearms etc.
5. Polyepoxide (epoxy): They are commonly used adhesive materials, also used for electrical components, and matrix of composite materials for binding the fillers along with hardenerd such as amine, amide and boron trifluoride.
6. Polyetheretherketone (PEEK): They are the class of most expensive commercial polymers and known for their excellent chemical and heat resistant nature along with biocompatibility which allows them for use in medical implants, aerospace moldings etc.
7. Polyetherimide (PEI): Chemically stable, high temperature resistant polymer which does not crystallise and is marketed as Ultem.
8. Polyimide: Class of amazing material performance polymers used in a varierty of products. Example is Kapton^{TM} tape.
9. Poly(lactic acid) (PLA): Most widely used biodegradable plastics made from lactic acid derived from fermentation of various agricultural products such as corn starch, or dairy products. A large number of aliphatic polyesters is mainly from poly(lactic acid). Used in disposable cups, plates, bowls etc.
10. Polymethyl methacrylate (PMMA) (acrylic): They are used in contact lenses, glazing materials (Perpex, Oroglas, Plexiglas are various trade names), fluorescent light diffusers, rear light covers for vehicles, etc. They are also quite popular as acrylic paints.
11. Polytetrafluoroethylene (PTFE): Example- Teflon. Used as heat-resistant, low friction coating materials, known as non-stick surfaces for frying pans, water slides, plumber’s tape.

12. Urea-formaldehyde (UF): Used as multi-colorable alternatives to phenolics in wood adhesives (for plywood, chip boards, hardboards) and electronic switchings.
13. Furan: Basically a resin from furfuryl alcohol, used in foundry sands and bio-derived composites.
14. Silicone: Because of the high-heat resistance, mainly used as sealant and in high temperature cooking utensil. Also used as a base resin in industrial paints.
15. Polysulfone: They are a class of high temperature melt resin which can easily be processed into membranes, filtration media, water heater dip tubes and other high temperature applications.

Cons of excessive use of platics. (Degradation and pollution)

Because of the excessive exploitation of polymers, the degradation of the polymeric materials has become a menace. The revolutionary success and dominance of plastics beginning from early 20th have led to social, economic and serious environmental concerns. The degradation of the plastic material is very slow owing to their chemical structure. The strong chemical bonds make them durable and resistant to most of the natural processes of degradation.

Figure 3: Plastic waste floating on the sea. (Source: European environment conference on plastic, 30 September 2013).

According to a report released by United Nations Environment Programme (UNEP), the plastic waste cost $ 13 billion in damages to the marine ecosystem. Entrapment of aquatic creatures like fishes, turtle in the plastic web produced by plastic ropes produce shocking pictures. The large debris of plastics near the beach destroy the scenic beauty and are often the breeding ground for mosquitoes or other micro-organisms and hence for epidemic diseases. It has a heavy toll on tourism industry also. Some of the apparent effects of plastic pollution with a short write up are as follows.

1. Disturbs the Food Chain

Since the plastic is available are almost all sizes, so they affect even the world's tiniest organisms such as planktons. The larger animals that are dependent on these small organisms for the food also gets infected or poisoned. This upsets the whole food chain. Man being the top at all the food chains suffers the most. The problem magnifies with each step further along the food chain. The toxin remain in the living organisms and destroys the health.

2. Groundwater pollution

When the large amount of toxins leak from the plastics and waste, they reach the ground water and destroys the quality of water. The rains distribute the wastes further. The environmental toxins percolate through the soil and reach ground water andf reservoirs and deteriorate the water quality. According to a survey, most of the litter and marine pollution is derived from plastics because of the ease of littering. Undoubtedly this has terrible effects on the marine species some of which are already extinct and others are on the verge of extinction. Eventually it affects human beings too.

3. Land pollution

Most of the plastic is simply dumped in landfills where it interacts with the moisture present in the soil or environment and releases hazardous toxins, which finally seep through underground and deteriorate the water quality. The water reservoirs which finally meet the oceans destroy the water quality in oceans too. Wind also carries lighter plastics from one place to another and hence increases the litter. Sometimes the plastics get stuck to the poles, trees, towers, ropes, walls, birds and animals which come in the vicinity and might choke them to death.

4. Air pollution

Most of the air pollution from plastics comes from the burning in open air. This leads to the environmental pollution because of the release of poisonous chemicals or toxins. Particulate matter produced as a result from plastic burning is inhaled by humans and animals which affect their health and increases respiratory problems or diseases.

5. High cost on economy

It also costs millions of dollars each year to clean the affected regions which are exposed to plastic menace. Open piles of plastic attract scavengers which further become the breeding ground of epidemic diseases. This causes a huge loss of flora and fauna. In the moden society, due to the increased prices of land, finding a place to dump the garbage is becoming a problem especially in densely populated areas.

6. Important species are getting extinct.

A large number of vulnerable species get extinct because of the harmful effects of plastic pollution. Plastics ingested by birds can obstruct and physically damage their digestive system or digestive ability and hence lead to malnutrition, starvation or ultimate death.

Global Efforts

The massive plastic pollution poses a great challenge to the world that requires an immediate global response. Firstly, the massive piles of plastic debris floating on the surface of the oceans destroying the scenic beauty of the beaches require immediate attention. The European Union should set up an exemplary strategy to set up the plastic waste policy. A second challenge lies in the conservation of resources. According to a survey, a large percentage of the plastic is still landfilled in the EU. Therefore, most of the precious resources are being wasted when they can be reused or recycled into the new products. Much of the energy is lost in the process. It requires a comprehensive policy response with stringent follow ups (53-56).

Plastic pollution existed because of the lack of awareness and ease of simply littering the plastics. According to an estimate, more than 200,000 tonnes of plastics in the form of micro-plastics is floating in the world's oceans. This posed a serious threat to the aquatic life and as a result, many of the vulnerable marine species have already extinct and some are on the verge of extinction. Moreover, the plastic enter the food chain and does more harm to the human beings who stand on the top of the food chain pyramid. Apart from that, the potential environmental hazard arising from the related phenomenon is beginning to be fully understood. As a result of growing number of reports about marine litter, plastic waster has started to attract increased attention than ever. The ease with which human beings simply litter plastics on the ground or in the water bodies like river, sea, drainage pipes resulted in the accumulation of plastic in the marine environment. According to a report, nearly 80 % of the plastics in the oceans are estimated to be coming from the land.

A number of countries have started taking this concern very seriously in order to save the earth from further disaster or ugliness. Developed countries like Japan encourage its people and authorities to segregate the plastic waste according to its type. A large portion of plastic is recycled or reused. Some plastics are prepared from bio-based sources and hence are easy to decompose naturally. National campaigns, messages through children in school and colleges, television and radio broadcast are some of the practices which promote the efficient use of plastics. Even debates, skits and competitions are organized in order to promote plastic waste management. These are some of the essential contributors which help achieve 'zero plastic to landfill' and move to a circular economy, which is very much the need of the hour. Plastics are essential in human's life. Infact, they are developed to ease the life. Plastic products and plastic waste are the two sides of the coin and are equally significant. In order to deal with the plastic menace, both bottom-up and bottom-down policies are to be approached. Then only, we can reach out to the problems at the grassroots and help solving it completely.

In order to tackle the problem globally, a stringent policies and implementation are need to be framed and all the countries are encouraged to participate in the plastic waste management. After all this is in the benefits of human beings and our earth.

1.2 *Biopolymers- Opportunities and Innovations*

One of the efficient ways to deal with the plastic pollution is through the use of biodegradable plastics which are equally good in material performance with an additional benefit of natural decomposition. These are considered to the sustainable option to tackle and curb the voracious demand and usage of plastic materials in almost all spheres of human life in its current form. The decomposition products of biodegradable plastics are simply water and carbon dioxide. No harmful chemicals or toxins are being released. In a way, they are pollution free. With the ever increasing demand of non-renewable resources, limited fossil fuels or the progressive decline of fossil resources, ever increasing cost of oil prices, and the heavy dependence on politically unstable countries for crude oil have initiated an increase in the search of alternative resources for the production of energy and useful chemicals. The shift is gradually towards replacement of fossil resources and use of bio based resources which are in abundance in nature, especially in the Asian region. Scientists are focusing the research in the development of polymeric materials and technology to prepare macromolecules based on renewable resources. Utilization of agricultural biomass has gained momentum. Scientists have successfully synthesized biofuels for instance from sugarcane or other crops by the process of fermentation. High performance materials can also be synthesized through bio-based resources with or without chemical modification.

Bioplastics are the future and sustainable solution. Bioplastics can be defined as the plastics which are mainly derived from renewable biomass resources, such as vegetable oils and fats, agricultural byproducts or crops such as corn, starch, or microbiota. Most of the commonly used plastics are derived from fossil fuel plastics which is petro-based. The production of such petro-based plastics requires large number of fossil fuels which generate a large quantity of greenhouse gases. In this way, the carbon footprint is very high (57-63). On the other hand, the production of bioplastics does not produce so much of greenhouse gases and have lesser carbon footprint or zero carbon footprints. While most of the bioplastics can be bio-degraded, some cannot be biodegraded. It depends on the chemical structure of the bioplastics being developed and the compatibility to the microorganisms. Bioplastics can be broken down to simpler substances, carbon dioxide and water mainly, in either aerobic environment or anaerobic environment. In terms of usage, the bioplastics are designed in such a way that some of them are able to replace the petro-based plastics in terms of material performance. They are being used in variety of applications such as in disposable items as in packaging, cups, plates, bowls, straws, cutlery, pots, bags, trays, fruits and vegetables containers, egg cartons, food packaging, bottles for soft drinks or beverages or dairy products. Other applications where durability is required include mobile phone casings, plastic pipes car interiors for insulation purpose, carpet fibres, and fuel lines. In many cased like these, the goal is to create items from sustainable resources, not towards biodegradability.

Ever since the discovery of poly(lactic acid) or PLA, a bio based plastics been made, scientists and researcher across the world have presented a lot of innovative items from PLA. For example, medical implants made from PLA emerged as a boon to large number of patients as it help them save a second operation, and is easy to dissolve in the body. It is also way too economical.

The definition of bio-based material has been explained by American Society for Testing and Materials (ASTM). According to ASTM, a bio-based material is:

"An organic material in which the carbon is derived from a renewable resource via biological processed. Such biobased materials include all the plants and animal mass derived mainly from CO_2 *recently fixed via process of photosynthesis, per definition of a renewable resource."*

Most of the widely used products in the market are made from a large number of natural feedstocks which includes corn, starch, rice palm fibres, wood cellulose, bagasse, potatotes, tapioca etc. The terms “biodegradable plastics” and “bio-based plastics” are not same and accordingly have different meaning. The biodegradable plastics are not necessarily bio-based plastics or vice versa. While some bio-based plastic products can biodegrade in municipal or commercial facilities, home composting or water bodies or aquatic environment, while others will only biodegrade in very specific environment. Some bio-based plastics don’t degrade at all. In many developed nations like in North America, special agencies dealing with plastic management have issued different certificates for products that are combustible or not despite having bio-based source in their manufacturing. According to BPI guidelines, for a product to be certified, it must be:

1. During the composting process, the product must disintegrate rapidly.
2. It must also biodegrade completely under the composting conditions.
3. Product must not reduce the utility or overall value of the finished compost. The humus manufactured during the process of composting will support plant life.
4. The product must not contain high amount of regulated metals.

Although bioplastics provide a sustainable solution and need to be promoted for usage worldwide, but they confronts several challenges despite benefits over petro-based plastics. Some of the challenges are described as:

1. The foremost challenge lies in their development and widespread acceptance. Scientists face challenges in developing new materials despite biomass abundance in nature. And moreover, the heavy dependence on petro-based plastics makes it difficult for the customers to substitute and use bioplastics.
2. There is also concern over the source for example use of genetically modified organisms (GMOs) in the production of bioplastics.
3. In order to make sure the balance between demand and supply, there is growing desire for sustainable growth of biomass which is difficult to predict based on physical and environmental factors.

4. Proper infrastructure and composting programs need to be developed and common people need to be aware of such programs.
5. There is also a concern for over contamination of recycling systems through the introduction of large variety of bioplastics.
6. Doubts on nanocomposites and blends of bioplastics with fossil fuels plastics.

   Proper planning and organized management with timely implementation can achieve all the goals and help overcome most of the challenges being put.

There are large numbers of bioplastics which are commercially available from:

1. Starch
2. Cellulose and cellulose acetate.
3. Lignin
4. Chitin and Chitosan
5. Polyhydroxyalkanoates
6. Polyesters from starch and sugars are popular and are being utilized in making utensils, and water resistant materials.Polyamides and polyolefin are also developed.
7. Poly(lactic acid): This is the most commonly used bioplastics. Its transparent property allows it touse in bottles, cups, candy wrappers, bags, clothing, sheets, towels, walls coverings. In the medical field, it is used in several implants, sutures, prosthetic materials, and also in materials for drug delivery as it is easily dissolved in the body.

Figure 4: Disposable cup from biopolymer, *poly(lactic acid).*

8. Protein based plastics.

In the initial phase, the bioplastics were expensive and hence were posed with challenges for consideration of substitutes over petroleum based plastics. However, over the period, the ease of the pricessing and lower temperatures required for their preparation along with the other factors like stable supply of biomass and high cost of petroleum or crude oil make the price of bioplastics more competitive with the regular plastics. There has been a considerable development in the field of bio-based plastics over few years and there is growing pressure on modern industry to promote the use of bio-plastics over the existing petro based plastics because of the various social, economic and environmental concerns. Although there are so much efforts being taken worldwide, yet they are not sufficient, and hence the responsibility is to be shared if we really want to tackle the problem of plastics.

1.3 ***Bio-based materials used in the present research***

The study and research on bio-based materials which can be degraded in natural environmental is necessary because of the considerable contribution to the green sustainable society based on low-carbonization and waster reuse. However, in practice, the studies are very difficult then they seem to be. So much efforts, resources and time are being devoted to develop a new high-performance bio-based polymer. Some of the developed bio-based polymers do not meet all the requirements. For example polylactides. Polylactides have low softening temperature of 60 °C, which restricts the usage as high-performance material. Moreover, the films from polylactides are easily deformed by immersing in hot water. From the structure property relationship, it is inferred that the presence of long flexible structure composed of aliphatic chains contribute towards low softening temperature. Based on this observation, Prof. Kaneko research group have established a new concept of molecular design which is based on ‘introduction of rigid aromatic component into the bio-based polymer backbone”. As a result, the material performance of the resulting polymer can be enhanced. Biodegradable plastics with high softening temperature such as over 150 °C were developed using the above concept (64-68).

The new bio-based polymers research has been published in Nature Materials, which is an highest impact factor journal. Further research continued in finding exotic bio-based sources for developing high performance polymers.

Polymeric materials offer numerous benefits over metallic materials. They are light weight, tough materials, recyclable, versatile, economical, and are widely available. Besides, they also contribute to lesser or zero carbon footprint. The use of bio-based materials is increasing in almost all the fields. Among exotic bio based sources, α-amino acids derived polymers are gaining attention. Prof. Kaneko research group has reported several high performance polymers based on α-amino acids and the studies have been published in high-impact journals. The α-amino acids based bio-polymers offer many advantages such as:

1. These polymers can improve the mechanical and thermal performance with aromatic components.
2. The degradation products are not toxic and can be easily decomposed.
3. They can be further modified to introduce new functionality.

1.3.1 *Aromatic amines as biomonomers*

Aromatic amines containing an amino-benzene or an aniline moiety comprise versatile natural and artificial compounds including bioactive molecules and resources for advanced materials. However, a bio-production platform has not been implemented. Aromatic amines that are characterized by an amino-substituted benzene (aniline) moiety (referred to hereinafter as AA) serve as resources from which to develop dyes, rubbers, plastics and conductive polymers, and they are important in a broad range of industries. Most living organisms produce the AA, 4-aminobenzoic acid, as a biosynthetic precursor of folate, which is an essential cofactor that is also a dietary supplement. Some AA are intermediates of antibacterial chloramphenicol, pristinamycin and other drugs, and developing a repertoire of AA is important from a pharmaceutical standpoint. Due to such substantial demand, various commercial AA have been synthesized by petroleum chemistry, whereas none has been derived from biomass, which limits the molecular design of practical bio-derived products based on AA.

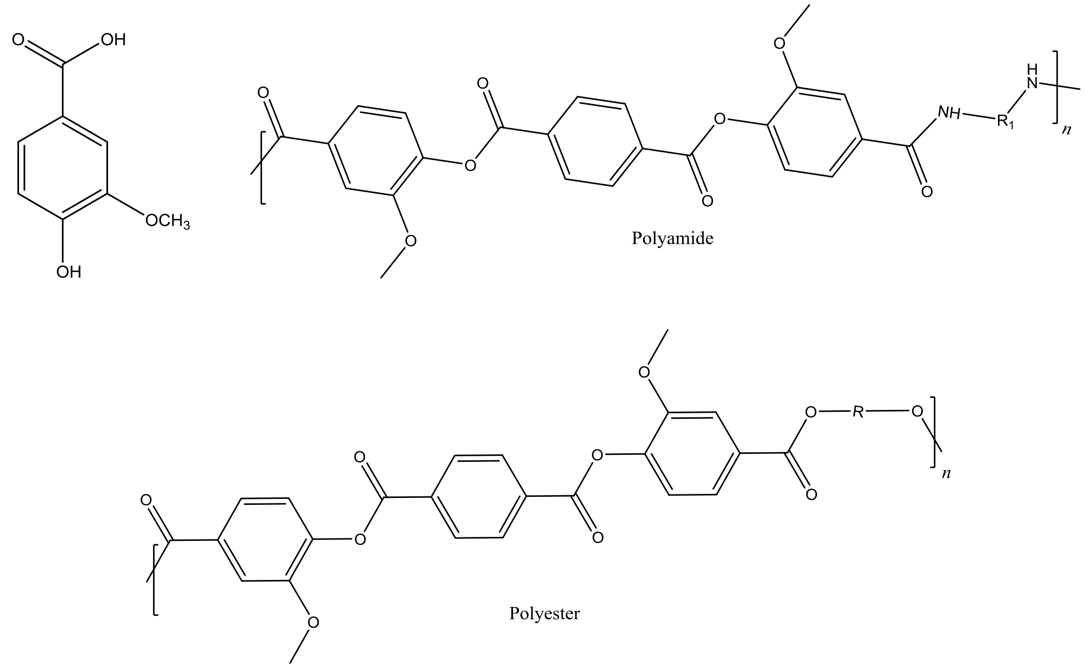
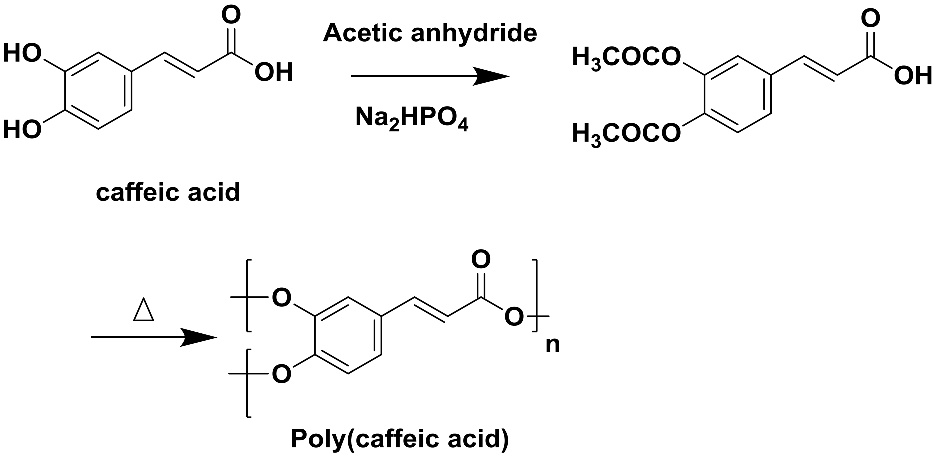
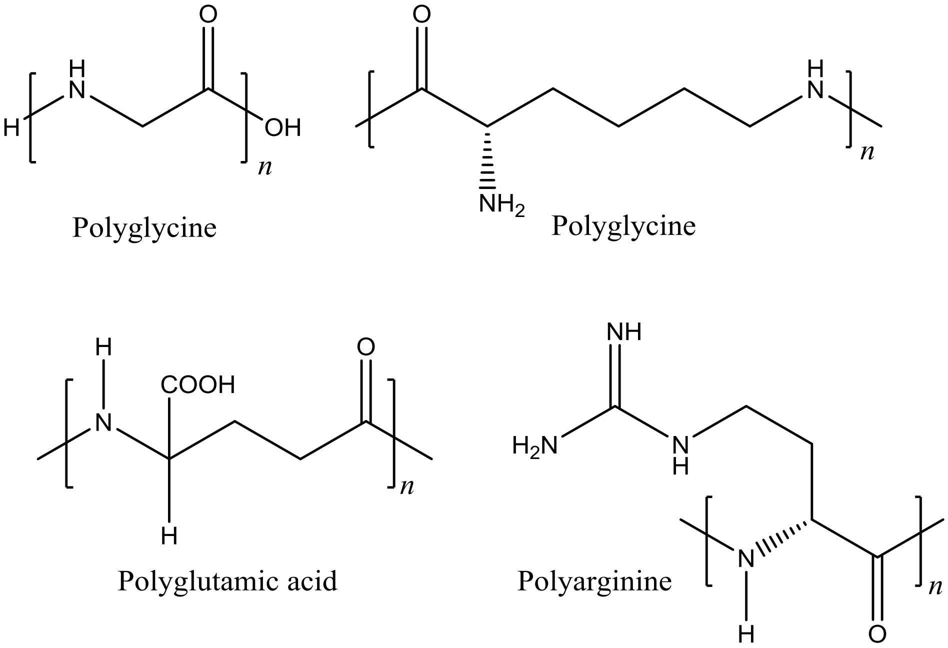


Figure 5: Chemical structure of vanillic acid, polyamide and polyester from vanillic acid.



Scheme 1. Synthetic route of polycondenstaion reaction of caffeic acid via acetylation for the preparation of polycaffeic acid.

Few decades earlier, bio derived aliphatic carboxylic acids were mainly used for developing biopolymers. For example, vanillic acid was used for making polyester and polyamide (Teijin Pharma, Japan), Caffeic acid and its derivatives were utilized to produce different kinds of polyesters and polyamides (Kaneko research group). However, the polymers developed have some limitations. They suffer from low thermal performance arising because of the flexible aliphatic regions.



Polyglycine

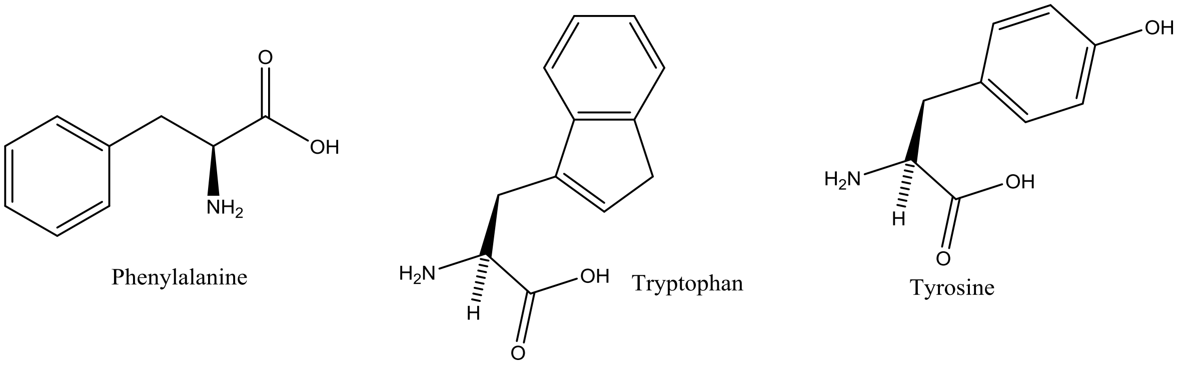
Polyglycine

Polyglutamic acid

Polyarginine

Figure 6: Biopolymers from naturally occurring amino acids.

Amino acids were promising materials for developing polymers as most of them are bioderived. Infact, the biopolymers from naturally occurring amino acid gained initial importance. A polyamino acid, sometimes also called polypeptide, is a synthetic biopolymer made from amino acid repeating units, i.e. -[NH-CHR-CO]x-. Polyamino acids are used in various medical and biological applications such: cell adhesion, drug delivery, gene therapy, diagnostic, oncology, antibacterial, antifungal, surface chemistry. Few of them were commercialized also. Still the thermomechanical properties of polyamino acids were not so high to consider them as high engineered plastics. Besides, they were easy to denaturate, and handling and storage of polyamino acids were difficult. However, aromatic amino acids have amazing potential as the rigid aromatic component was one of the pre-requisite for high-performance plastics. Aromatic amines are significant in the production of advanced polymer materials including functional and/or high-performance plastics. The amine group and the aromatic moiety of AA induce nucleophilic reactivity and excellent thermomechanical performance, respectively. Aromatic amines are polycondensed with carbonyl compounds to generate aromatic polyamides, polyimides, polyazoles, polyurea and polyazomethines. When polycondensed with aromatic acids, AA generate super-engineering plastics with extremely high thermomechanical properties. These include poly(*p*-phenylene terephthalamide) (Kevlar^{TM}) and poly(4,4′-oxydiphenylene pyromellitimide) (Kapton^{TM}) that serve as thermostable materials in fabric for body armor and other flame-retardant materials, fiber-reinforced plastics for electronic devices, vehicle bodies and anti-pressure cylinders. The applications of super-engineered plastics are diversifying, and this is increasing the annual global production of AA-derived plastics to around 100,000 tons. Global production of aromatic polyamides accounts for several hundreds of millions of US dollars, which indicates the size of the contribution of AA to both the economy and society.



Phenylalanine

Tryptophan

Tyrosine

Figure 7: Naturally occurring aromatic amino acids.

Polymers developed by Prof. Kaneko research group

With the objective of the development of high-performance bio-based polymers which are indispensable for the establishment of a green sustainable society, our research group has developed some of the unconventional alternative bio-based polymers from exotic amino acids and other sources. Few of the bio-based materials used are hydroxycinnamate derivatives such as *p*-coumaric acid (4-hydroxycinnamic acid) and caffeic acid (3,4-dihydroxycinnamic acid) (68) for polyacrylate design. The production of aromatic polyamides and polyimides requires aromatic carboxylic acids and/or AA as building blocks. Whereas aromatic carboxylic acids (such as terephthalic acid) have been derived from biomass, AA have not, and this has precluded the development of fully bio-oriented aromatic polyamides and polyimides. Our recent microbial production of the non-natural AA, 4-aminocinnamic acid (4ACA), from 4-aminophenylalanine (4APhe), which is an intermediate of the chloramphenicol and pristinamycin biosynthesis pathway, followed by synthesis of ultra-high-performance polyimide is the exception. Not only 4ACA, but also other AA derived from biomass would serve as innovative monomers for synthesizing bio-AA plastics, and their environmental impact should be enormous, considering that they would replace polyamides and polyimides derived in bulk from petroleum.

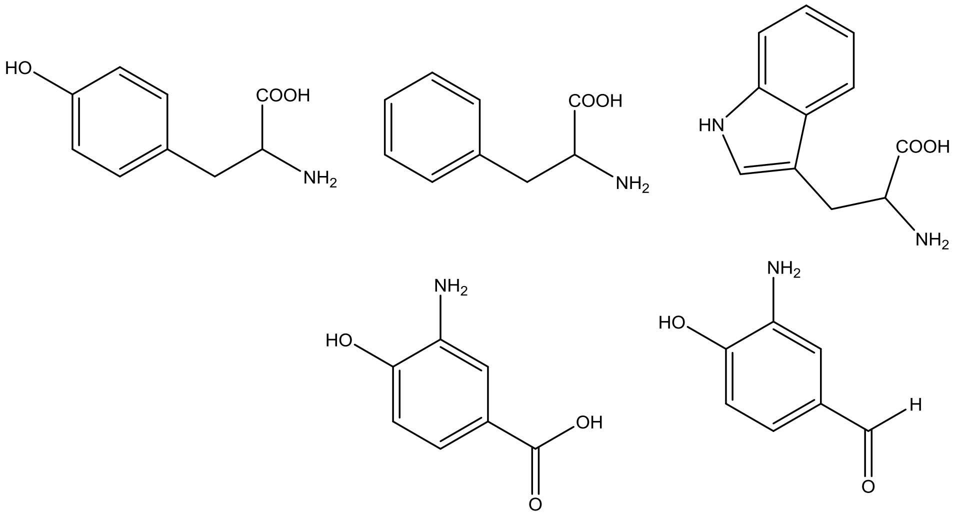


Figure 8: structures of various aromatic amino acids (used by Kaneko research group).

Our research group have reported on the bio-based aromatic diamine 4,4’-diaminotruxillic acid (4ATA) that was photonically derived from microorganismal 4-aminocinnamic acid (4ACA) produced by a bioconversion using PAL enzyme from 4-aminophenylalanine

(4APhe) which was fermented by genetically-manipulated *Escherichia coli*, as a bio-based aromatic diamine monomer. On the other hand, some production steps of bioconversion and photodimerization can be omitted if 4APhe having two amines is used as a diamine monomer for aromatic bio-based polymers with high thermal and mechanical performances. Bioplastics such as polyureas, polyamide and polyimide (69-71) developed from 4ATA showed ultrahigh thermal and mechanical performances.

1.3.2 *Bio-production of 4-aminophenylalanine*

Production of 4APhe. The Rhizobium etli gxrA gene (accession number, ACO35311.1) was amplifies by PCR using the primers (5´-CCGGATCCATGTCAGTTCGTCCTCCCGTCC-3´ and 5´-GCGAATTCCTAATAACCGGCGGCGCGATCG-3´), digested with BamHI and EcoRI, and then cloned into BamHI + EcoRIdigested pCWfoxy vectors. The 2.5-kb DNA fragments containing lacI and gxrA were amplified using the primers (5´-GCGAATTCCAGTCGGGAAACCTGTCGTGCC-3´ and 5´-CCGTATGCTAATAACCGGCGGCGCGATCG-3´), and the resulting plasmid was digested with EcoRI and SphI and cloned into EcoRI + SphI-digested pHSG298 (Takara Bio, Kyoto, Japan) to generate pHSGgxrA. The AtPal4 cDNA (NP_187645.1) was amplified using the primers (5´-CCGGATCCATGGAGCTATGCAATCAAAACAATC-3´ and 5´-CCGCATGCTCAACAGATTGAAACCGGAGCTCCG-3´), digested with BamHI and SphI, and then cloned into BamHI +SphI-dugested pHSGgxrA to give rise to pHSG-Atpal4. Escherichia coli NST3717 transformed with pHSG-Atpal4 was cultured in 100 mL of Luria broth (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl) containing 50 mg/L Kanamycin sulfate at 37 °C and 120 rpm. After the optical density of the culture reached 3,1 mM IPTG was added and incubated for another 12 h under the same conditions. The cells were collected by centrifugation, washed with 0.1 M potassium phosphate (pH 8.0), and incubated in the same buffer containing 10 mM 4-APhe at 37 °C and 120 rom for 12 h. The reactions were analyzed by HPLC (HP-1100, Hewlett-Packard, CA, USA) using a packed silica gel column (Purospher star RP-18e 5 µm, 4.6 × 150 mm, Merck, Germany).

Methanol: 20 mM phosphate (6:4 v/v) was used as the eluant at a flow rate of 8.0 mL/min. Phenylalanine ammonia lyase activity was measured as described previously.

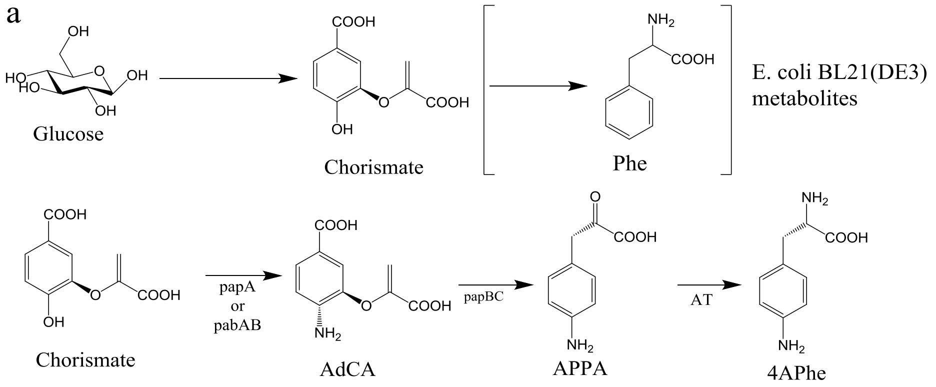


Figure 9: Biosynthetic pathway for the production of 4-APhe using *E.coli.*

One research has showed that some bacteria produced 4-aminophenylalanine (4APhe) as an intermediate of antibiotics (72). There are established systems for fermenting glucose biomass to produce 4APhe (73-75).

Further, 4-aminocinnamic acid could also be produced from 4-APhe.

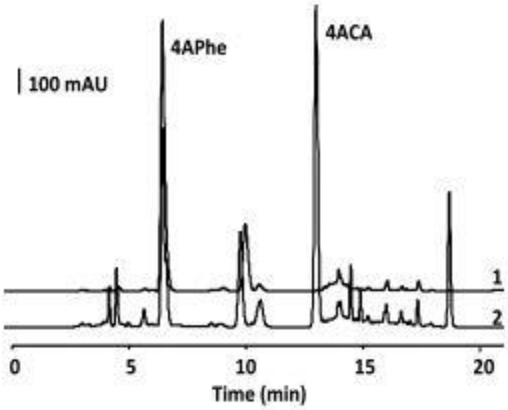


Figure 10: High-performance liquid chromatography (HPLC) separation of 4ACA bioconverted from 4APhe. The AtPal4 producing recombinant E. coli was incubated with 10 mM 4APhe at 37 °C for 0 h (line1) and 12 h (line 2).

In the present research, I describe the syntheses and properties of aromatic bio-based polyureas (PUs), and polyimides (PIs) from microorganism-derived diamine, 4APhe, by a polyaddition with various aromatic diisocyanates, and dianhydrides respectively. Some of bio-based PUs and PIs prepared here show high glass transition temperature around 200 ^{o}C and mechanical strengths over 100 MPa. Hence, these PUs and PIs might be potentially used in sustainable plastics, coating materials. Further, this approach presents unconventional alternative methods for producing high-performance bio-based polymers which might potentially contribute to the green and sustainable society.

1.3.3 *High performance polymers*

High performance polymers exhibit exceptional stability upon exposure to extreme environments and have properties that surpass those of traditional polymers. These materials are defined in many ways depending upon the application, and to some extent, on the organized systems used for developing or employing the materials. Outstanding thermal resistance and/ or mechanical strength, low specific density, high-conductivity, high-thermal, electrical, or sound insulation properties or superior flame resistance were few characteristics to determine high performance polymers. Most of the factors that contributed to high-performance and heat resistance properties of these polymers are presented as: resonance stabilization, primary bond strength, molecular symmetry, secondary bonding forces, molecular weight and distribution, rigid intra-chain structure, cross linking, mechanism of bond-cleavage, and additives or reinforcements (fillers, clays, or miscellaneous nanoparticles). Because of the superior performance of these materials, the demand of high-performance materials is always surging. In my research, I focus on two kinds of high-performance polymers, polyureas and polyimides.

1.3.4 *Polyureas*

According to the definition, a polyurea is elastomer mainly derived from the reaction product of isocyanate and a synthetic resin blend monomers (e.g. diamine) through step growth polymerization. The two components can be aliphatic, aromatic or mixed in nature depending upon the material performance required. Polyurea is a polymer which contains monomers combined through urea linkage. Interestingly, the first organic compound discovered is urea which has the chemical formula $(NH_2)_2CO$. In polyurea, the alternating monomer units of diisocyanates and diamines react with each other to form urea linkages. Originally, polyurea was developed to protect tabletop edges. Further research by Mark S Barton and Mark Schlitcher (US 5534295) led to the development of two component polyurethane and polyurea spray elastomers. The noteworthy features such as fast reactivity, relative moisture insensitivity, weather resistant, chemical and thermal resistant, broad spectrum of color availability made them useful coating materials on large surface area projects such as manhole and tunnel coating, large buildings, swimming pools, truck bed liners etc. Furthermore, through proper primer and surface treatment, the excellent adhesion to concrete and steel is obtained. And hence, it revolutionized the coating industry. They can be applied through various ways or methods such as spray molding, ink jet etc. Very fast curing time allows many coatings to be built up quickly. Some polyureas are reported to reach strengths of 6000 psi (40 MPa) tensile and more than 500 % elongation which make them a very tough coating material (76). For shipping industry, polyurea coating came as a boon as their coatings last for 25-30 years unlike bitumen coatings lasting for 3-4 years. Besides usage as coating materials, the copolymers of polyurea and polyurethane has also been used for developing Spandex^{TM} which is a textile material mainly used in slim suits or as body shaper. Spandex^{TM} is known for its exceptional elasticity and durability and hence has replaced natural rubber in a variety of products.

Figure 11: Spandex's body suits or slim body shaper made of polyurea.

In a world of increasing environmental awareness, polyurea proves to be an effective and economical choice for governments and businesses for their elastomeric and structural needs. Material improvements in cure times, hardness and fire retardancy are being made every day. Application equipment and spray tip innovations are being introduced more rapidly than ever to meet the demand for better, more efficient means of getting the product sprayed in place. Copolymers of polyurea and polyurethane are used in the manufacture of spandex$^{\text{TM}}$, which was invented in 1959.

According to one citation report (Figure 12 and 13), there are very few published articles under the polyurea research theme. And the number of citations is increasing every year. Therefore, polyurea is one of the interesting areas of polymer domain. The number of papers published under the topic bio-polyureas is even negligible. So there is a large scope of scientific research under the theme biopolyureas. Results found: 1076

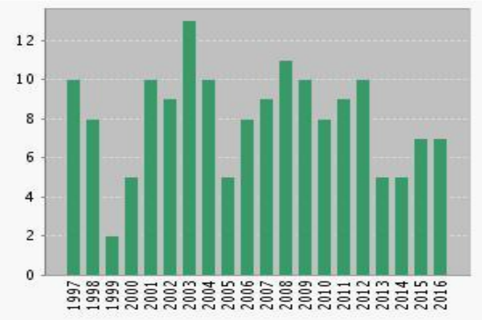


Figure 12: Published Items in each year from 1990 till date January 18, 2017.

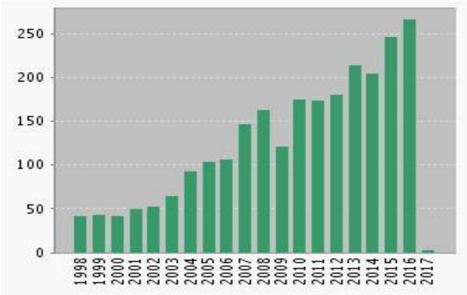


Figure 13: Citations in each year from 1990 till date January 18, 2017.

(Citation Report: 206 (from Web of Science Core Collection) TOPIC: (polyureas). Refined by: WEB OF SCIENCE CATEGORIES: (POLYMER SCIENCE) AND TOPIC: (polyureas). Timespan: All years. Indexes: SCI-EXPANDED, SSCI, A&HCI, ESCI.

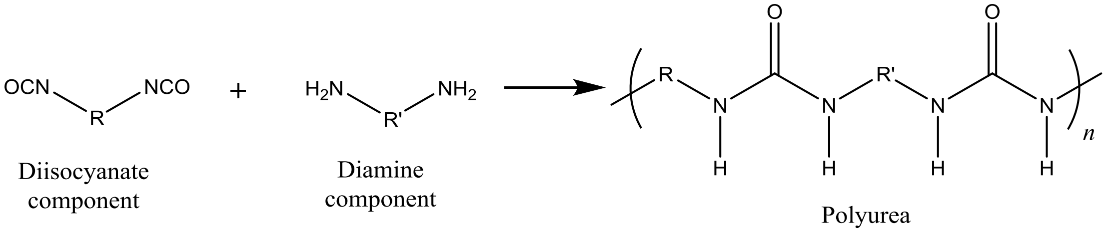
Sum of the Times Cited: 15648, Sum of Times Cited without self-citations: 12368

Citing Articles: 10554, Citing Articles without self-citations: 9815, Average Citations per Item: 14.54, h-index: 55

Polyureas are very useful materials and hence can be used in a broad range of applications because of their physical and chemical properties. The following section highlights few of the advantages of polyureas.

1. Fast cure: The curing time of polyureas is very fast. They are used as coating materials on the roads because of this feature.
2. Temperature and water insensitivity: polyureas are temperature and water insensitive. Sudden variations in weather conditions like humidity, temperature, etc. have low effect.
3. 100 % Solids: Fast reaction between two components render one coating in 1:1 volume ratio, and hence multi coating is not required. In this way, polyurea material is ecofriendly.
4. Excellent physical properties: The tensile strength, tear strength and elongation at break of polyurea materials are high which makes them tough material.
5. High heat resistance: Polyureas are thermally stable even upto 150 °C or above in most cases.
6. Pigment compatibility: in order to enhance light stability and change appearance, colorants are added to polyureas during curing process.
7. Formulation stability: They can be applied in various methods and hence can be formulated in soft to hard elastomers.
8. Composites: Being reinforcible materials, they are compatible with fillers and fibers.

By selecting the appropriate isocyanate component and amine blend, a variety of system reactivities is possible. They could be aliphatic or aromatic. Usually, in order to have good thermal and mechanical properties, aromatic components are selected.



Scheme2: General scheme illustrating the two main components, diisocyanates and diamines to synthesize polyurea.

In my research, I developed a series of polyureas using 4-aminophenylalanine (4-APhe) and various diisocyanates such as MDI, MMDI, 1,3-PDI, 1,4-PDI, TDI.

1.3.5 *Polyimides*

Polyimides (also abbreviated as PI) are a class of high-performance polymers. With their exceptionally high heat resistance, polyimides have been commonly used in a range of rugged materials, e.g. high temperature fuel cells, displays, and for military equipments since 1955. A classical example of polyimide is Kapton^{TM}, which is produced by condensation of pyromellitic dianhydride and 4,4’-oxydianiline.

Several methods are possible to prepare polyimides, among them:

1. The reaction between a dianhydride and diamine, which is also the most commonly used method.
2. The reaction between dianhydride and a diisocyanate.

The intermediate poly(amic acid) is usually converted to the final polyimide by the thermal imidization route. Heating the poly(amic acid) mixture to 100 °C and holding for one hour, heating from 100 °C to 200 °C and holding for one hour, heating from 200 °C to 300 °C and holding for one hour and slow cooling to the room temperature from 300 °C.

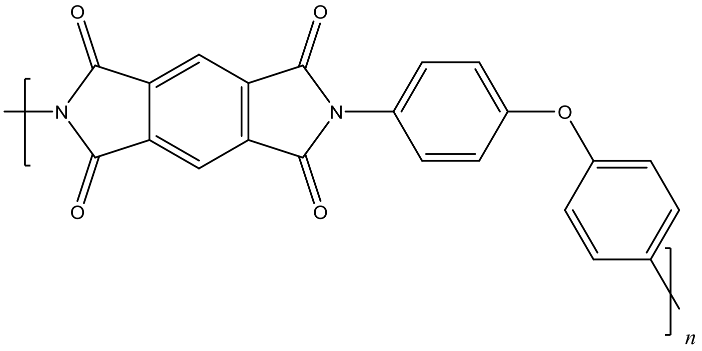


Figure 14: Chemical structure of commercially available polyimide, Kapton^{TM}.

Hundreds of diamines and dianhydrides have been examined to tune the physical and especially the processing properties of these materials. Polyimides materials tend to be insoluble and have high softening temperatures, arising from charge-transfer interactions between the planar subunits.

Polyimide materials are lightweight, flexible, resistant to heat and chemicals. Hence, they are widely used in high temperature plastics, adhesives, dielectrics, photoresists, nonlinear optical materials, membrane materials for separation, and Langmuir–Blodgett (LB) films, among others. Additionally, polyimides are used in a diverse range of applications, including the fields of aerospace, defense, and opto-electronics; they are also used in liquid crystal alignments, composites, electroluminescent devices, electrochromic materials, polymer electrolyte fuel cells, polymer memories, fiber optics, etc. (77-80). The chemistry of polyimides relies on its monomers, diamines and dianhydrides. Any variation in monomers allowed the researchers to tune the properties and applications of polyimides. However bio-derived PIs were very difficult to prepare since the aromatic diamines cannot be made using biosynthesis because they are incompatible with microorganisms and plant cells, presumably due to the combined interactions of ionic, hydrophobic, and π- electron-related factors with cell constituents. Even aromatic monoamines have rarely been produced by microorganisms. Understanding the structure-property relationship in polyimides simplify the understanding of applications exhibited by them. The reaction for polyimide syntheses is expected to depend upon the electrophilicity of the carbonyl groups of the dianhydride and the nucleophilicity of the amino nitrogen atom of the diamine. Electrophilicity of the dianhydrides is usually gauged in terms of electron affinity (Ea) which is further measured by polarographic techniques. The following section highlights a small account of the role of dianhydrides.

Dianhydrides

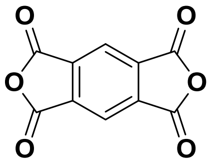
Dianhydrides are widely used as monomers for polyimides. They offer numerous advantages:

1. Dianhydrides offer versatility when used on their own or in admixtures with other dianhydrides.
2. They are compatible with all diamines for building traditional polymers or new hybrids.
3. They are commercially available in high purity and variable particle size to meet specific requirements.
4. Fabricated parts which require high thermal resistance usually rely on dianhydrides for higher T_g.
5. For optimal processing of polyimides, the dianhydrides are available in variable pot life.
6. Another advantage also includes low mix viscosity for easier incorporation of fillers, fibers and additives.

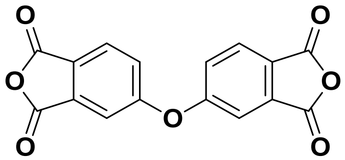
Different aromatic dianhydrides used in the project are:

1. **PMDA or Pyromellitic Dianhydride:** PMDA is a commonly used dianhydride monomer that is used to prepare many commercial polyimides such as Kapton^{TM}. PMDA-based polyimides are insoluble in organic solvents. Thus, they must be processed in the form of their soluble polyamic acid precursors. PMDA that has the

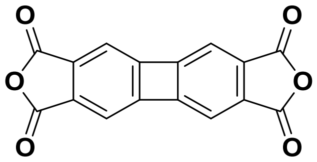
highest E_a, of the common aromatic diamines also usually demonstrates the highest reactivity when reacted with different diamines.



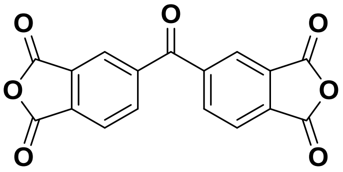
2. **OPDA or 4,4'-Oxydiphthalic dianhydride:** ODPA is a monomer that can be incorporated in polyimides to increase their flexibility and solubility while maintaining their thermal stability.



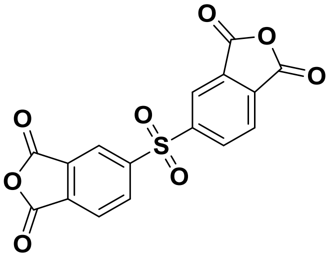
3. **BPDA or 3,3',4,4'-Biphenyltetracarboxylic acid dianhydride:** Mainly employed for commercial polyimides. The asymmetric BPDA is a monomer that can be incorporated in polyimides to reduce their melt viscosity and increase their *T*g.



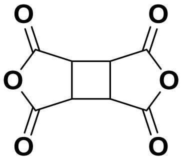
4. **BTDA or 3,3',4,4'-Benzophenonetetracarboxylic dianhydride:** BTDA is important monomer of polyimide, and used for production of polyimide material. The material has high temperature resistance, low temperature resistance, corrosion resistance, radiation resistance, insulation, shock resistance, excellent performance. Can be made into structure parts, laminates, films, adhesives, coatings, insulation materials and reinforcing material, etc., can be widely used in the field of aerospace, electrical / electronics, shipbuilding, automobile, precision machinery.



5. **DSDA or 3,3',4,4'-diphenylsulfonetetracarboxylic dianhydride:** Mainly used as a reagent for High-Performance polyimides polymer research.



6. **CBDA or 1,2,3,4-Cyclobutanetetracarboxylic dianhydride:** CBDA is widely used as a raw material for polyimide resins or polyamic acid resins in the application of alignment films for liquid-crystal-display devices. In addition, polymer material made of CBDA offers unparalleled transparency, excellent heat resistance and flexibility.



In my research, I developed a series of polyimides using 4-aminophenylalanine (4-APhe) and various dianhydrides such as CMDA, PMDA, BPDA, BTDA, OPDA, and BSDA.

1.4 *Aim and scope of the study*

My research activities are based on following purposes.

1. Introducing exotic bio-based amino acid as diamine monomer.

2. Development of high performance bioplastics such as Polyureas and polyimides using the above monomer.
3. Investigating thermo-mechanical properties of these polymers and clarifying the structure-property relationships.
4. Further modification in polymeric structure to enhance the applicability.

Main work involves:

1. *Syntheses and characterization of bio-polyureas from exotic amino acid as diamine monomer with aromatic ring in the polymeric backbone*
2. *Syntheses and characterization of unconventional alternative biopolyimides from functionalized aromatic amino acid.*
3. *Reinforcement with carbon fibres to make CFRP (carbon fibre reinforced polymers).*
4. *Cell culturing on polymeric films.*

1.5 *Research novelty*

To my best knowledge, this is the first attempt to utilize exotic α-amino acid, 4-APhe, as monomeric entity to develop high-performance biopolymers.

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Chapter 2

Syntheses and characterization of aromatic polyureas from 4-aminophenylalanine as diamino acid monomer

Chapter 2

2.1 Introduction

In a world of increasing environmental awareness, polyurea (1-6) proves to be an effective and economical choice for governments and businesses for their elastomeric and structural needs. By selecting the appropriate isocyanate component and amine blend, a variety of system reactivities is possible. They could be aliphatic or aromatic. Usually, in order to have good thermal and mechanical properties, aromatic components are selected. Recently, as the cost of petroleum increases, making products with biobased materials (7-11) is increasingly attractive. Making the transition from a petroleum-based to a bio-based economy also gives us an opportunity for product standards to ensure that impacts on the environment, health, and society are included (12-22). Researchers across the world have invested extensive scientific research and development in order to create the next-generation of bio based polymers which might help reduce the use of non-renewable fossil fuels without reducing the performance of materials. The thermal and mechanical performance of currently-available bioplastics such as poly(lactic acid) (23), poly(hydroxyalkanoate) (24), and poly(butylene succinate) (25) from bio-available aliphatic molecules is quite low, which restrict their commercial or industrial usage (26-29). On the other hand, the introduction of aromatic rings in the backbone of polymer chain boosts their thermal and mechanical performances and widens their application fields into electronic parts, automobiles parts, and optical materials (30-32). Under such social and scientific backgrounds, we have reported aromatic bio-based polymers from hydroxycinnamate derivatives such as *p*-coumaric acid (4-hydroxycinnamic acid) (33, 34), caffeic acid (3,4-dihydroxycinnamic acid) (35, 36), and ferulic acid (3-methoxy-4-hydroxycinnamic acid) (37) for polyarylate design. Moreover high-performance bio-based polyureas (38), polyamides (39, 40), and polyimides (41-43) have been developed using a photodimer of 4-aminocinnamic acid (4ACA) produced by a bioconversion using PAL enzyme from 4-aminophenylalanine (4APhe) which was fermented by genetically-manipulated *Escherichia coli*, as a bio-based aromatic diamine monomer (44, 45). On the other hand, some production steps of bioconversion and photodimerization can be omitted if 4APhe having two amines is used as a diamine monomer for aromatic bio-based polymers with high thermal and mechanical performances.

Here we describe the syntheses and properties of aromatic bio-based polyureas (PUs) from microorganism-derived diamine, 4APhe, by a polyaddition with various aromatic diisocyanates. Some of bio-based PUs prepared here show high glass transition temperature around 200 ^{o}C and mechanical strengths over 100 MPa. Hence, these PUs might be potentially used in sustainable plastics, coating materials.

2.2 Experimental

2.2.1 Materials

4-APhe hydrochloride salt used as a bio-based material for the PU syntheses was purchased from Watanabe Chemical Ind. Ltd. (Tokyo, Japan). Sodium hydroxide (NaOH; 97 % purity, Kanto Chemical, Tokyo, Japan) used for neutralization of 4APhe hydrochloride salt was used as received. Trimethylsilyl chloride (TMSCl; Sigma-Aldrich LLC, Tokyo, Japan), and Methanol (MeOH; super dehydrated grade, Wako Pure Chemical Industries Ltd., Japan), Dehydrated N-methyl-2-pyrrolidone (NMP; 99.0 % purity, Kanto Chemical, Tokyo, Japan), N, N-dimethylacetamide (DMAc; 99.8 % purity, Wako, Tokyo, Japan) used as solvents were used without further purification. Diisocyanate monomers such as 4,4’-diphenylmethane diisocyanate (MDI; 98 % purity, TCI, Tokyo, Japan), 4,4’-diisocyanato-3,3’-dimethyldiphenylmethane (MMDI, 98 % purity, TCI, Tokyo, Japan), 1,3-phenyl diisocyanate (98 % purity, TCI, Tokyo, Japan), 1,4-phenyl diisocyanate (98 % purity, TCI, Tokyo, Japan), 2,4-toluene diisocyanate (99 % purity, TCI, Tokyo, Japan) were used as received.

2.2.2 Measurements

Nuclear magnetic resonance (NMR) measurements such as ^{1}H-NMR and ^{13}C-NMR were performed by Bruker biospin AG 400 MHz, 54 mm spectrometer using DMSO-d_6 as a solvent. Specimen with a concentration of 0.5 mM in DMSO-d_6 in NMR test tubes of borosilicate glass (Wako Pure Chemical Industries, Φ 4.932-4.970 × 7 in.) was used.

The Fourier-transformed infrared (FT-IR) spectra were recorded with a Perkin-Elmer Spectrum One spectrometer in a wavenumber range between 4000 and 600 cm^{-1} using a diamond-attenuated total reflection (ATR) accessory.

The mass spectra were measured using a Fourier-transformed ion cyclotron resonance mass spectrometer (FT-ICR MS, Solarix) with an external ESI source (Analytica of Branford Inc.). The sample solution was infused into the ESI source in a positive ion mode with a syringe pump at 60 mL/h and was desolvated by a countercurrent nitrogen gas heated to 250 ^oC, and scanned from m/z 150 to m/z 1000.

X-ray diffraction (XRD) was made using monochromated CuKa beam (λ = 0.15418 nm) with an acceleration voltage of 40 kV (30 mA) by a 2θ scanning from 5 to 80 degree in a reflection mode using rotor X-ray emitter (RINT 2000; Rigaku Smart Lab) was used to determine the degree of crystallization using the Gram AI program.

The tensile measurements were carried out at an elongation speed of 0.5 mm/min on a tensiometer (Instron 3365, Kawasaki, Japan) at room temperature using polymer rectangle film with a length of 3 cm, a width of 1 cm and a thickness of 5 µm.

The number average molecular weight (*M*n), weight average molecular weight (*M*w) and molecular weight distribution (PDI) of the polymers were determined by gel permeation chromatography (GPC; Shodex GPC-101 with a tandem connection column system of KD-803 and KD-807 (Shodex, Tokyo, Japan)) after calibration with pullulan standards in dimethylformamide (DMF) as eluent at a flow rate of 0.5 ml min^{-1} using the RI signal detector (shodex, Tokyo, Japan). The concentration of sample used was 5 g/L, filtered after stirring overnight.

The ultraviolet-visible (UV-vis) transmission spectra were recorded by Perkin Elmer, Lambda 25 UV/Vis spectrometer at room temperature over the range of 200-800 nm in a quartz cell (1 cm × 1 cm) under Deuterium and Halogen lamp as light source. The solubility of the polymers was investigated using 1 mg of sample in 1mL of solvent at room temperature or at 50 °C.

Differential scanning calorimetry (DSC) of dried polymer samples (5-8 mg) was carried out under a nitrogen atmosphere at a scanning rate of 10 ^{o}C/min between 0 and 200 ^{o}C (Seiko Instruments SII, X-DSC7000T).

Thermogravimetry (TGA; SSC/5200 SII Seiko Instruments Inc.) was carried out to determine weight-loss temperature by heating from 30 to 800 °C at a rate of 5 °C/min under a nitrogen atmosphere. The polymer specimens for these thermal measurements were dried at 150 °C for 1 h to remove absorbed moisture and remained solvents before measurements.

2.2.3 Syntheses

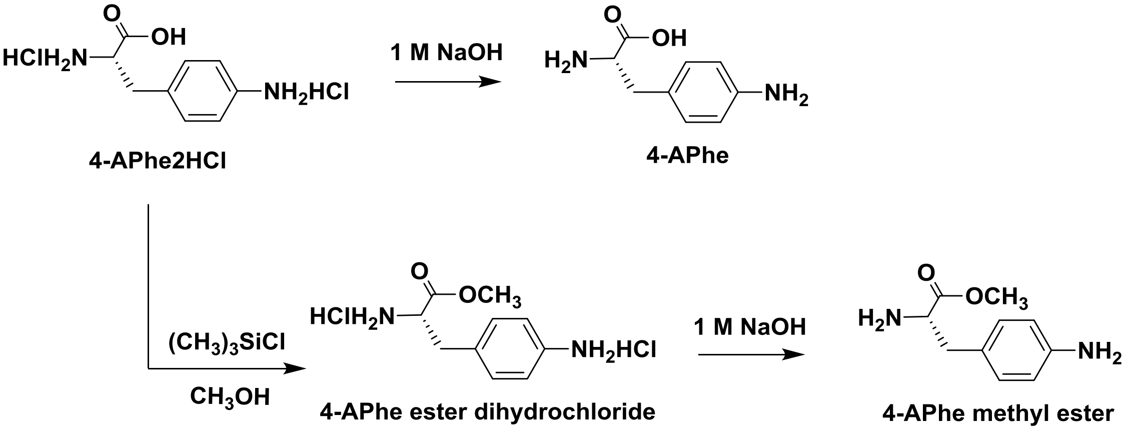
2.2.3.1 Monomer preparation

Synthesis of 4-aminophenylalanine (4-APhe).

4-APhe was synthesized (Scheme 1) by neutralization of 4-aminophenylalanine dihydrochloride by the procedure below. 4-APhe.2HCl (5.0 g, 19.8 mmol) was dissolved in 100 mL of ethanol to prepare white suspension. 1 M NaOH aq (40 mL, 40.0 mmol) was added dropwise into the suspension at 0 °C to turn into colorless homogeneous solution. By the addition of more NaOH aq, white precipitates of 4-APhe appeared over pH 9. The white precipitates of 4-APhe formed are separated by filtration and dried in vacuo overnight at temperature 60 °C (yield: 2.1 g, 59 %). The product was highly hygroscopic.

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.60-2.66 (dd, 1H, β-methylene), 2.94-2.98 (dd, 1H, α-methylene), 3.20-3.23 (water peak), 4.89 (s, 2H, amino), 6.46-6.48 (d, 2H, ortho-hydrogens of phenylene), 6.88-6.90 (d, 2H, meta-hydrogens of phenylene).

^{13}C-NMR (100 MHz, DMSO-d_6, δ, ppm): 35.5, 56.0, 116.8, 125.5, 130.2, 145.4, 174.1. FT-ICR MS (ESI) calcd for [M-H$^+$, $C_9H_{11}N_2O_2$]$^-$: calculated value 179.0826, observed value 179.0825.



Scheme 2.1. Syntheses of 4-aminophenylalanine and 4-aminophenylalanine methyl ester.

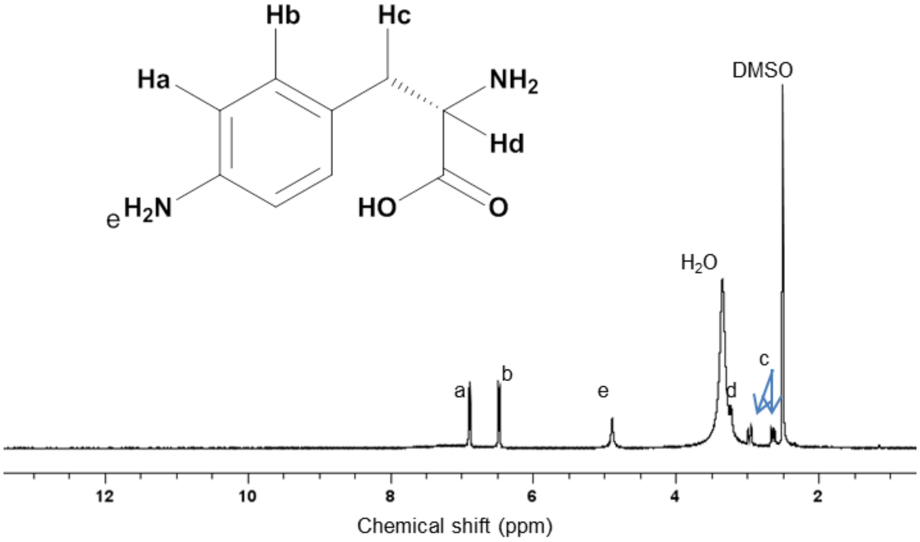


Figure 4. ^{1}H NMR spectrum of 4-aminophenylalanine.

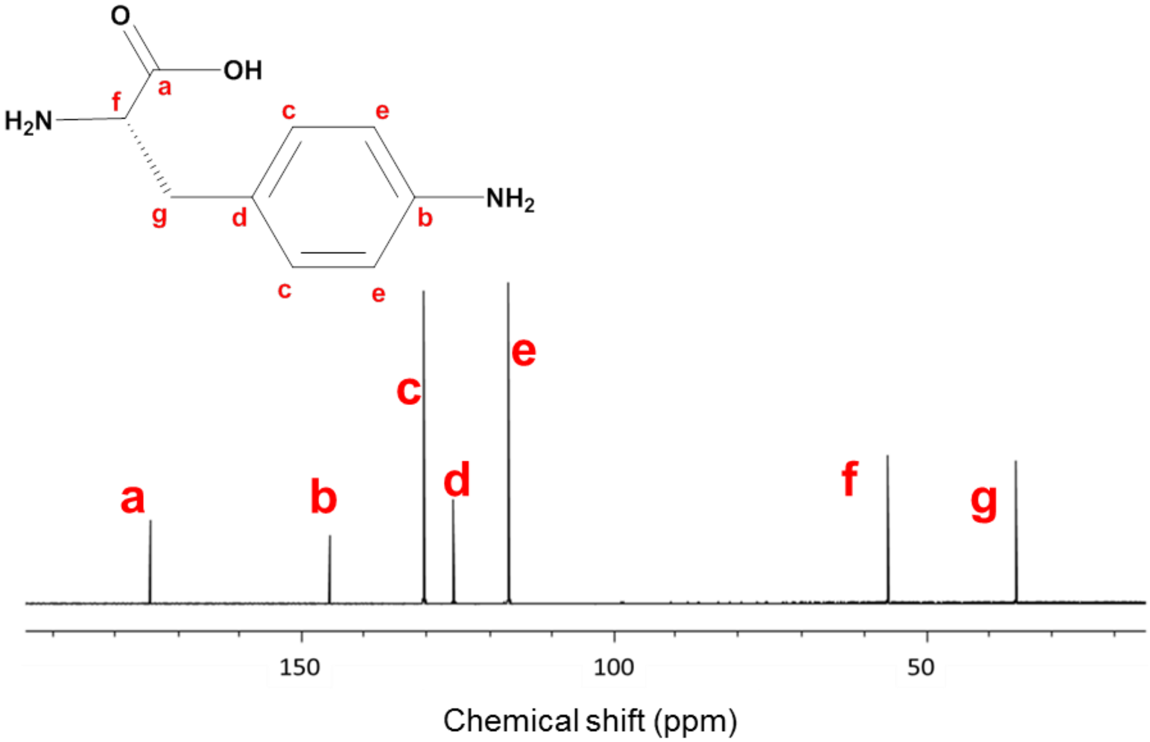


Figure 5. ^{13}C-NMR spectrum of 4-aminophenylalanine.

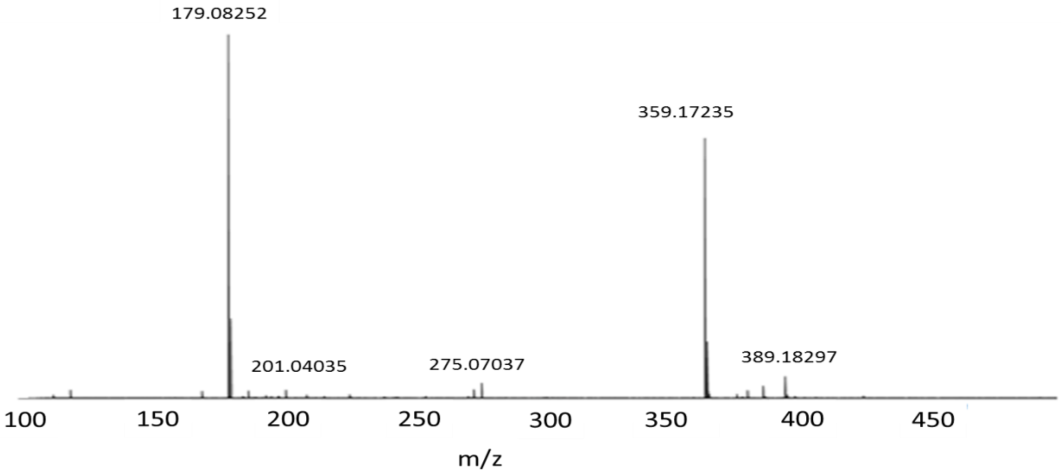


Figure 6. Mass spectrum of 4-aminophenylalanine.

Synthesis of 4-aminophenylalanine methyl ester.

Methyl ester of 4-aminophenylalanine is synthesized (scheme 1) by the procedure as follows: 4-APhe dihydrochloride (3.00 g, 11.8 mmol) was mixed with trimethylsilyl chloride (TMSCl) (4.5 mL, 35.57 mmol) in 15 mL of methanol in a round-bottomed flask under a nitrogen atmosphere at room temperature. The reaction proceeded in the formation of white precipitates. The reaction mixture was dissolved in water, and extracted by ethyl acetate. The ethyl acetate organic layer was dried over anhydrous $MgSO_4$, and it was then evaporated and neutralized using 1 N NaOH. The specifications of methyl ester of 4-APhe are as follows:

Yield: 2.7 g, **% yield:** 89 %.

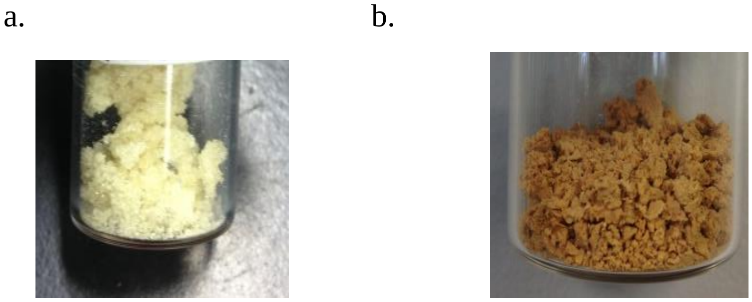


Figure 7. Physical appearance of monomer a). Me-4-APhe.2HCl and b). Me-APhe.

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.62 (dd, 1H, methylene), 2.67 (dd, 1H, methylene), 3.56 (s, 3H, methyl), 4.87 (s, 2H, NH_2), 6.46 (d, 2H, aromatic ring), 6.79-6.85 (d, 2H, aromatic ring).

^{13}C-NMR (100 MHz, DMSO-d_6, δ, ppm): 35.4, 49.0, 53.7, 119.2, 128.7, 130.8, 139.7, 170.6.

Table1: Solubilities of 4-APhe.2HCl and Me-4-APhe in organic solvents at room temperature; ‘+’ represents completely soluble, ‘-’ represents insolubility and ‘±’ represents partial solubility.

Monomer ➡ **Solvent**	**4APhe.2HCl**	**4-APhe**	**Me-APhe**
Water	+	+	+
Methanol	+	+	+
Ethanol	+	±	±
Acetonitrile	-	-	-
tetrahydrofuran	-	-	-
Diethylether	-	-	-
DMF	+	+	+
DMAc	+	+	+
NMP	+	+	+
DMSO	±	+	±
Ethyl acetate	-	-	-
Chloroform	-	-	-
Hexane	-	-	-
Conc. H_2SO_4	+	+	+

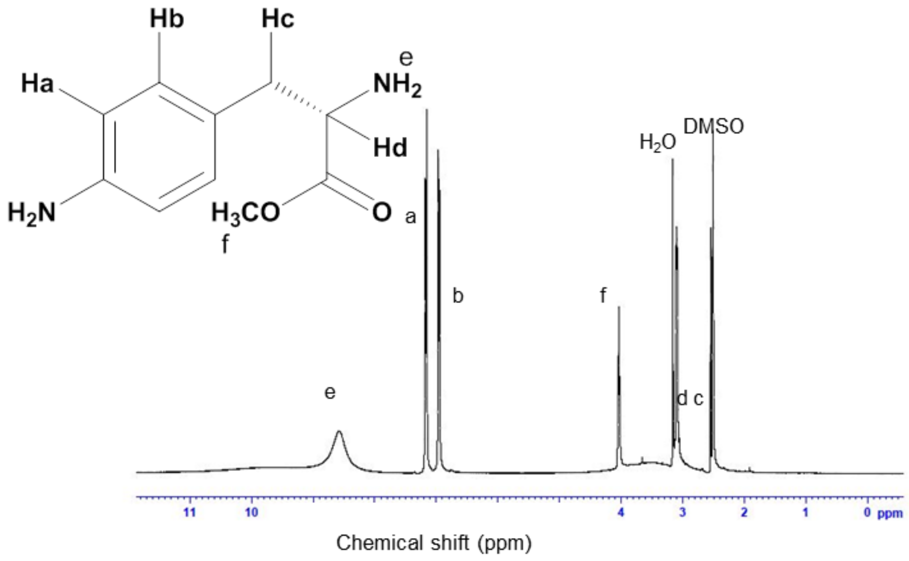


Figure 8. ^{1}H NMR spectrum of methyl ester of 4-aminophenylalanine.

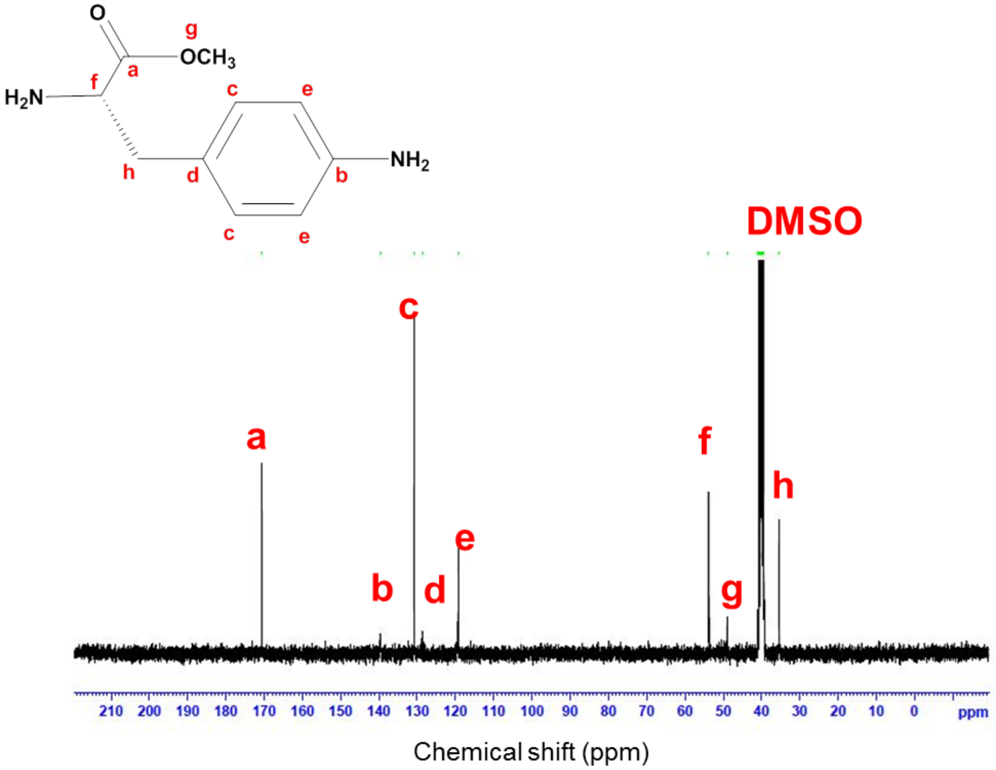
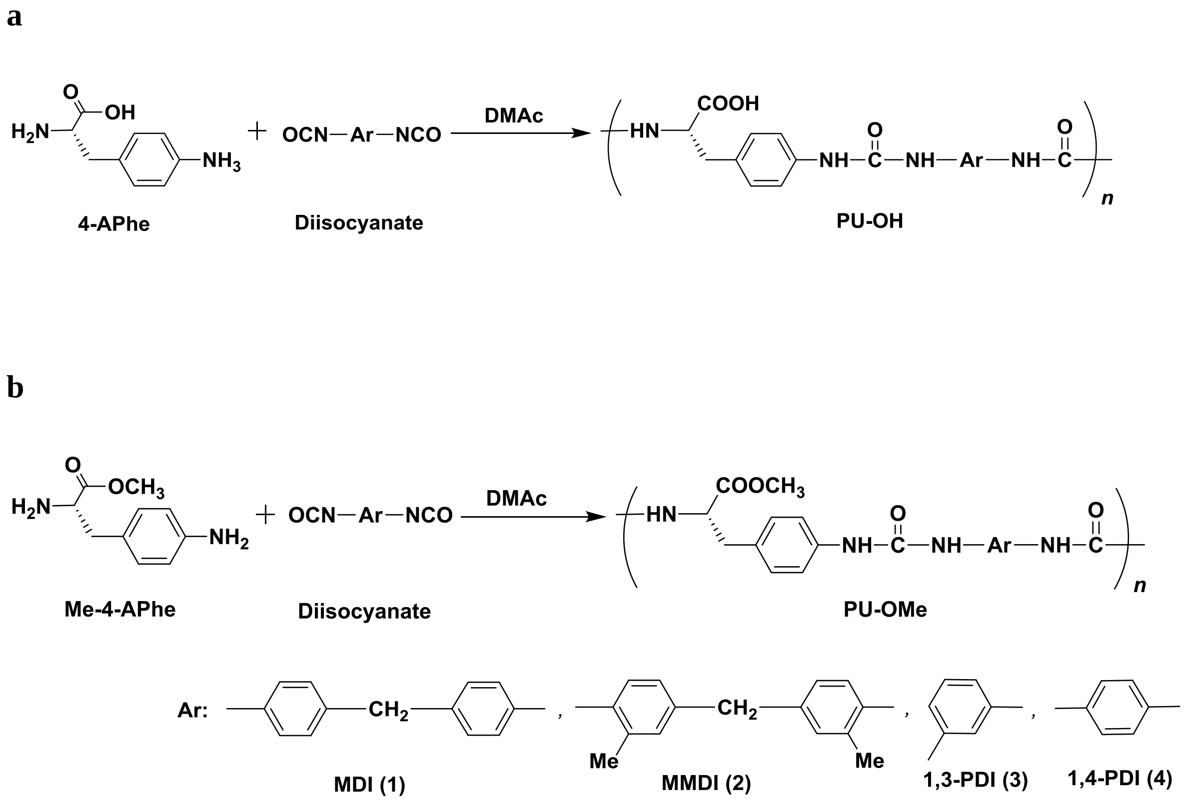


Figure 9. ^{13}C-NMR spectrum of Methyl ester of 4-aminophenylalanine.



Scheme 2.2. Syntheses of bio-based aromatic polyureas derived from 4-aminophenylalanine and 4-aminophenylalanine methyl ester as diamine monomers. Ar means aromatic moiety drawn at bottom where structures are numbered from 1 to 4.

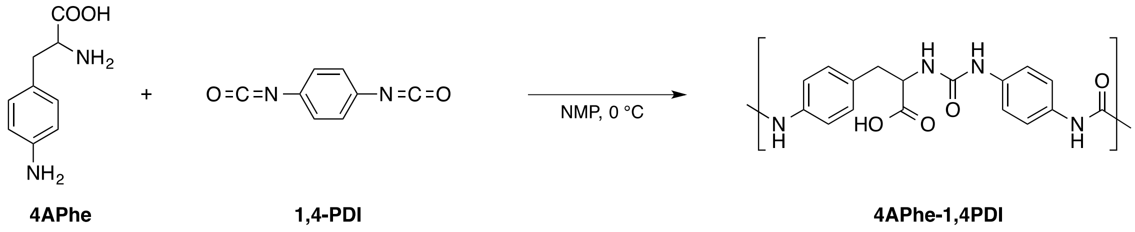
2.2.3.2 Polyureas syntheses

Syntheses of polyureas. A series of polyureas (PUs) were synthesized by a reaction of diamine, 4-APhe or 4-Aphe methyl ester with equimolar amounts of diisocyanates as shown in scheme 2. Representative procedures for the syntheses of PUs are shown as follows. 4APhe (500 mg, 2.78 mmol) or 4APhe methyl ester (500 mg, 2.57 mmol) was dissolved in NMP (5 mL) in 10 mL reaction flask under nitrogen atmosphere. 4,4'-diphenylmethane diisocyanate (MDI, 772 mg, 2.78 mmol was added into the diamine solution with constant stirring at room temperature and the reaction solution became gradually viscous. After polymerization for 24 hours, the viscous reaction solution was diluted by NMP to thin appropriately, and then poured into water or methanol to precipitate solid fibrils of PU. The fibrils were collected by filtration, washed with the methanol and dried in *vacuo*. Representative picture of PU fiber is shown in Figure 2. The targeted structure of the PU was confirmed by ^{1}H-NMR. The specifications are as follows. ^{1}H-NMR (400 MHz, DMSO-d6): δ 2.96-3.01 (d, 2H, methylene), 3.76 (s, 2H, methylene), 4.37-4.42 (dd, 1H, methine), 6.22-6.23 (d, 1H, urea linkage), 7.00-7.36 (m, 12H, aromatic ring), 8.59-8.62 (d, 3H, urea linkage);^{13}C-NMR (100 MHz, DMSO-d6): δ 36.8, 39.5, 53.7, 128.9, 129.6, 130.3, 134.4, 135.0, 137.7, 138.1, 138.3, 152.6, 154.7, 173.7.

Molecular weights of PUs were determined by gel permeation chromatography (GPC) and listed in Table 1 along with the yields. The weight-average molecular weights (M_w) ranged above 1.63×10^5 and high enough to analyze them as materials. PUs films were obtained by spin-casting over DMF yellow solution onto silicon wafer or aluminum plate (spin rate = 150 rpm for 5 min, MS-A100 Spincoater, Mikasa Co., Ltd.) and successively dried at room temperature. Other PUs were synthesized by polyaddition of 4APhe or 4Aphe methyl ester with diisocyanate monomers such as 4,4'-diisocyanate-3,3'-dimethyldiphenylmethane (MMDI, 694 mg, 2.78 mmol), 1,4-phenylene diisocyanate (1,4-PDI, 440 mg, 2.78 mmol), 1,3-phenylene diisocyanate (1,3-PDI, 440 mg, 2.78 mmol), and toluene diisocyanate (TDI, 400 mg, 2.57 mmol) by the analogous procedures with the case of MDI.

A detailed procedure with relevant characterizations for each polymer is explained as below.

1. Synthesis of polyurea (4APhe-1, 4PDI) using 1,4-phenylene diisocyanate



Scheme 2.3: Reaction scheme for the synthesis of polyurea (4-APhe-1,4PDI).

Procedure

NMP (5.0 ml) was added to 4APhe (500 mg, 2.78 mmol) under nitrogen atmosphere and the mixture was stirred under ice cooling. Then 1,4-phenylene diisocyanate (1,4-PDI, 440 mg, 2.78 mmol) was added, and the mixture was stirred for 40 hours. The reaction solution was added dropwise to distilled water to re-precipitate the polymer. The precipitate was filtered, washed with methanol and vacuum dried to obtain white fibrillar polyurea (4APhe-1, 4PDI).

Yield: 992 mg, % **yield:** 100%

Number average molecular weight M_n: 1.53 x 10^6

Weight average molecular weight M_w: 1.71 x 10^6

^{1}H-NMR (400 MHz, DMSO-d6): δ 2.90-2.97 (d, 2H, METHYLENE), 4.39 (s, 1H, METHYLENE), 6.18-6.21 (t, 1H, UREA), 7.08-7.34 (m, Aromatic ring), 8.47-8.57 (t, 3H, UREA).

^{13}C NMR (100 MHz, DMSO-d6): δ 36.8, 53.7, 118.1, 118.9, 129.6, 130.2, 134.2, 138.5, 152.7, 154.8, 173.8.

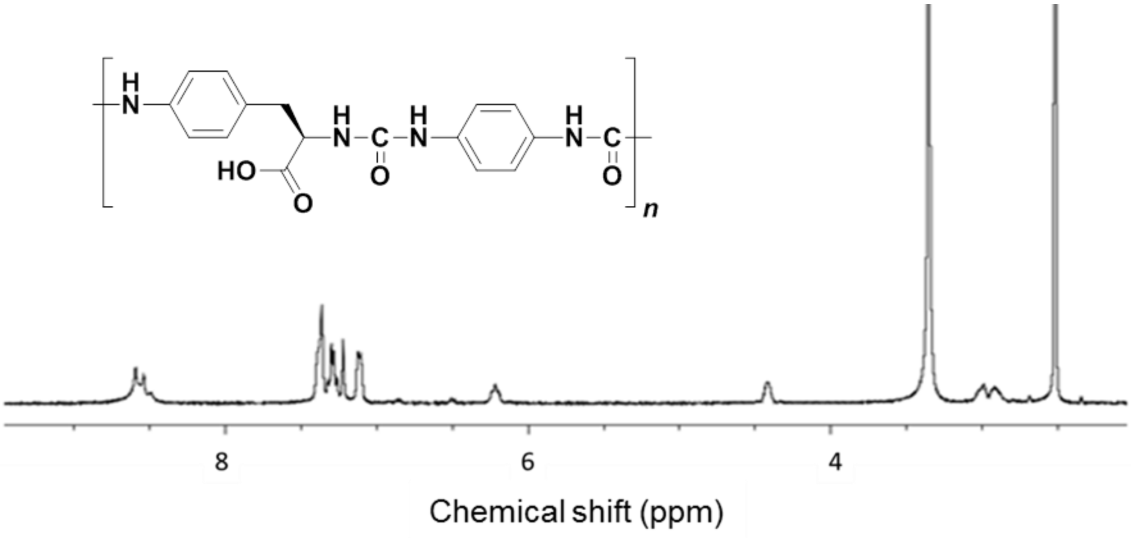


Figure 10. ^{1}H NMR spectrum of polyurea (4APhe-1, 4-PDI).

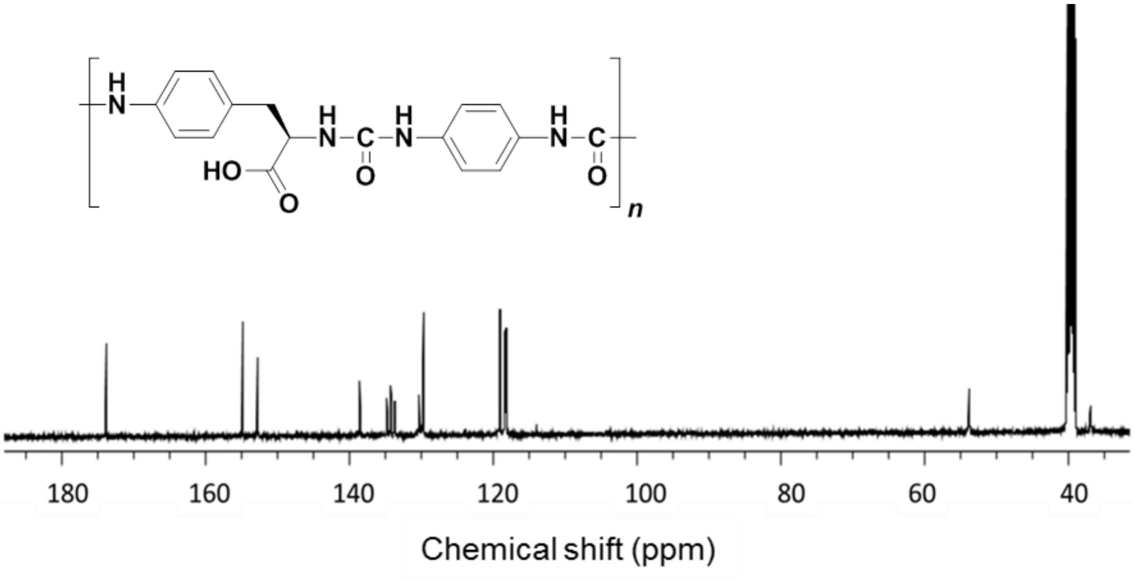


Figure 11.^{13}C NMR spectrum of polyurea (4APhe-1, 4-PDI).

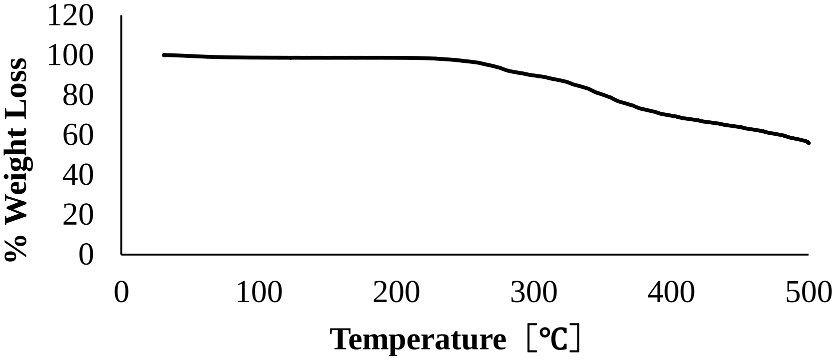
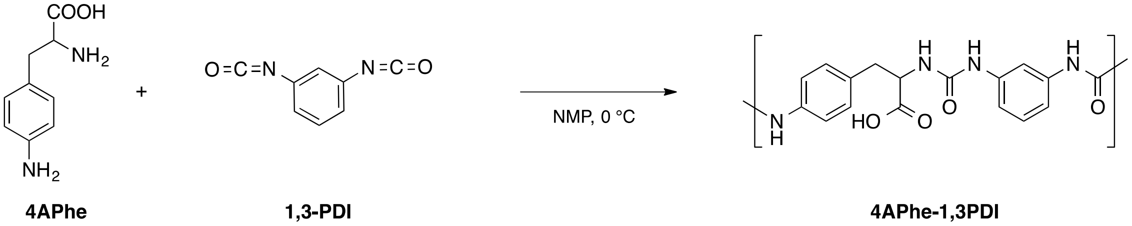


Figure 12. TGA curve polyurea (4-Aphe-1,4PDI).

2. **Synthesis of polyurea (4APhe-1, 3PDI) using 1,3-phenylene diisocyanate**



Scheme 2.4: Reaction scheme for the synthesis of polyurea (4-APhe-1,3PDI).

Procedure

Under nitrogen atmosphere, NMP (5.0 ml) was added to 4APhe (500 mg, 2.78 mmol), and the mixture was stirred under ice cooling. Then 1,3-phenylene diisocyanate (1,3-PDI, 440 mg, 2.78 mmol) was added and stirred for 40 hours, and the reaction solution was added dropwise to distilled water to reprecipitate the polymer. The precipitate was filtered, washed with methanol and vacuum dried to obtain white fibril-like polyurea (4APhe-1, 3PDI).

Yield: 680 mg, **Yield:** 72%

Number average molecular weight M_n: 1.22×10^{6},

Weight average molecular weight M_w: 1.42×10^{6}

^{1}H-NMR (400 MHz, DMSO-d6): δ 2.89-2.99 (d, 2H, METHYLENE), 4.40 (s, 1H, METHYLENE), 6.21-6.26 (t, 1H, Aromatic ring), 8.58-8.73 (dd, 3H, Urea).

^{13}C NMR (100 MHz, DMSO-d6): δ 36.8, 53.7, 107.2, 111.1, 118.1, 129.6, 138.3, 140.2, 140.6, 152.5, 154.6, 173.7.

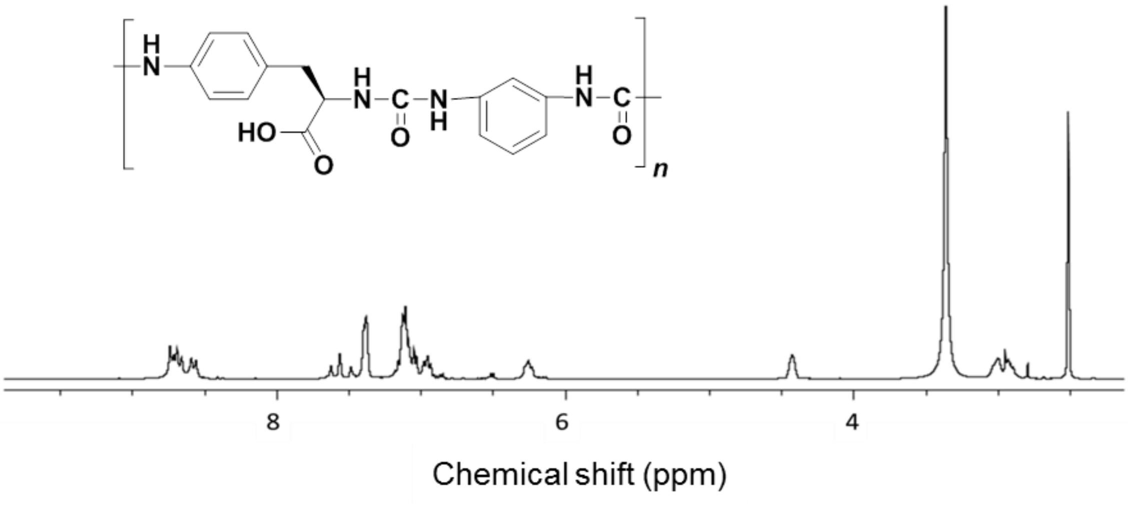


Figure 13. ^{1}H NMR spectrum of polyurea (4APhe-1,3-PDI).

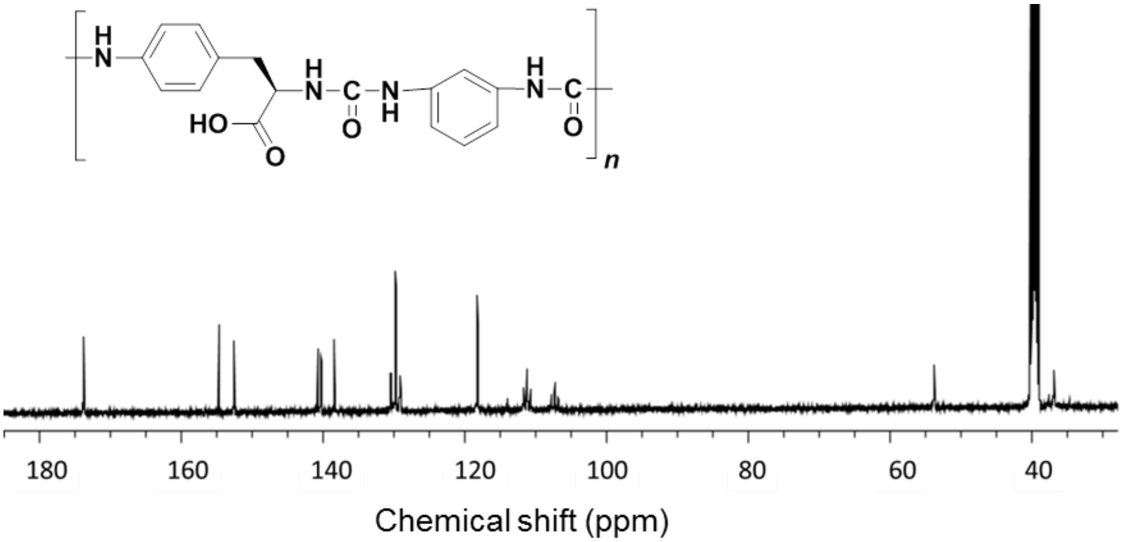


Figure 14. ^{13}C NMR spectrum of polyurea (4APhe-1,3-PDI).

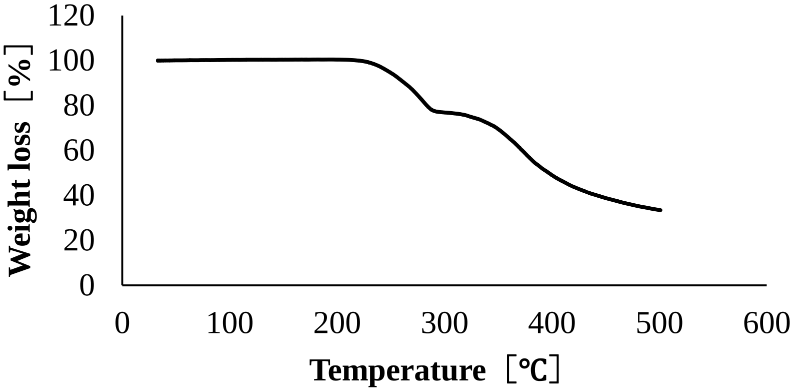
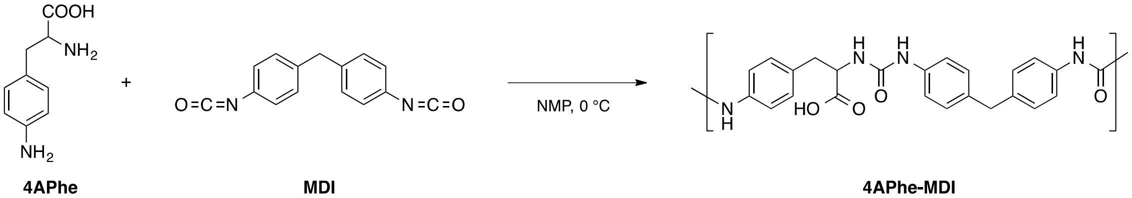


Figure 15. TGA curve of polyurea (4APhe-1,3-PDI).

3. Synthesis of polyurea (4APhe-MDI) using 4,4-diphenylmethane diisocyanate



Scheme 2.5: Reaction scheme for the synthesis of polyurea (4-APhe-MDI).

Procedure

Under nitrogen atmosphere, NMP (6.0 ml) was added to 4APhe (500 mg, 2.78 mmol) and the mixture was stirred under ice cooling. Next, 4,4'-diphenylmethane diisocyanate (MDI, 772 mg, 2.78 mmol) was added and stirred for 41 hours, and the reaction solution was dropped into distilled water to reprecipitate the polymer. The precipitate was filtered, washed with methanol and vacuum dried to obtain white fibrillar polyurea (4APhe-MDI).

Yield: 1.18 g, **yield:** 94%

Number average molecular weight M_n: 1.84 x 10^6,

Weight average molecular weight M_w: 2.69 x 10^6

1H-NMR (400 MHz, DMSO-d6): δ 2.96-3.01 (d, 2H, METHYLENE), 3.76 (s, 2H, METHYLENE), 4.37-4.42 (dd, 1H, METHYLENE), 6.22-6.23 , urea), 7.00-7.36 (m, 12H, aromatic ring), 8.59-8.62 (d, 3H, urea).

13C NMR (100 MHz, DMSO-d6): δ 36.8, 39.5, 53.7, 128.9, 129.6, 130.3, 134.4, 135.0, 137.7, 138.1, 138.3, 152.6, 154.7, 173.7.

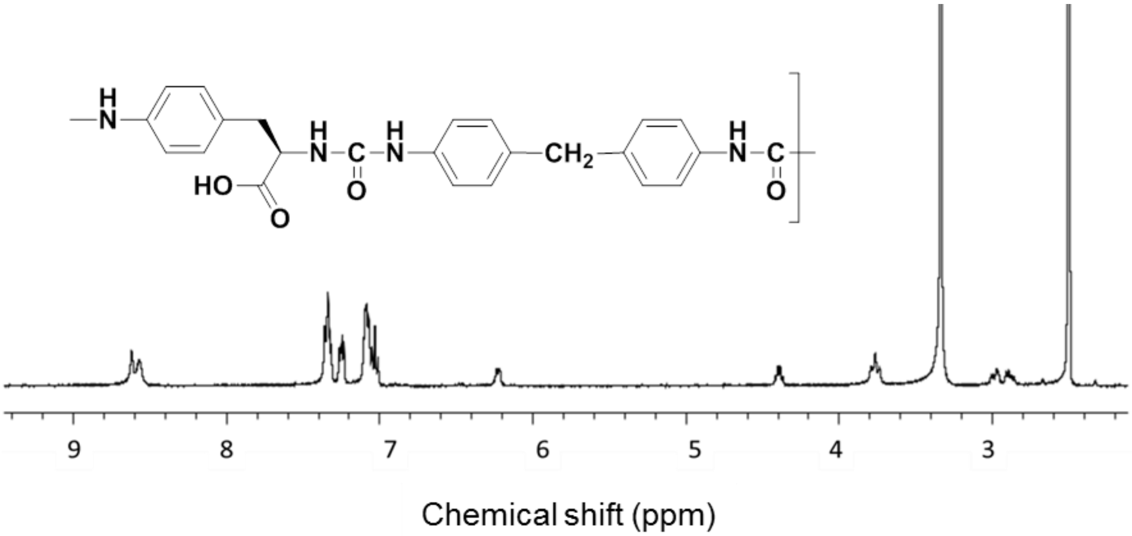


Figure 16. ^{1}H NMR spectrum of polyurea (4 APhe-MDI).

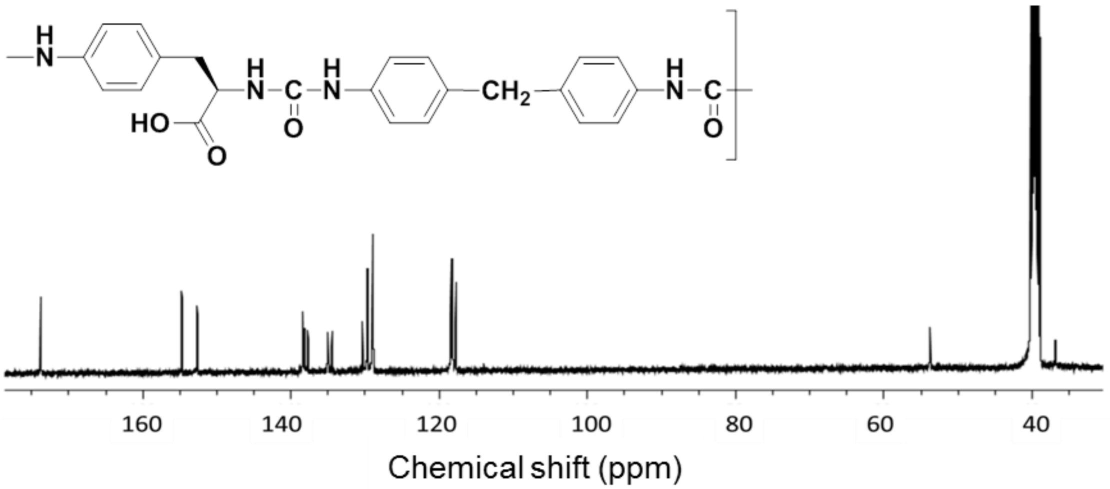


Figure 17. ^{13}C NMR spectrum of polyurea (4APhe-MDI).

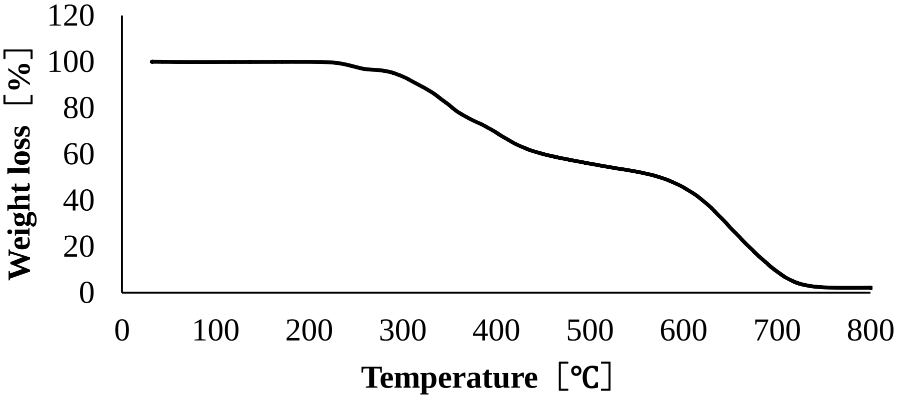
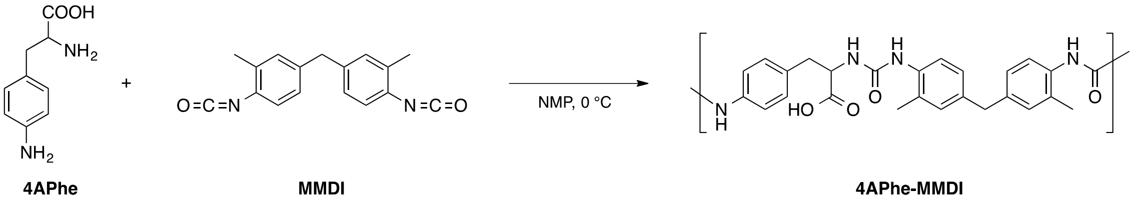


Figure 18. TGA curve of polyurea (4APhe-MDI).

4. Synthesis of polyurea (4APhe-MMDI) using 4,4'-diisocyanate-3,3'-dimethyldiphenylmethane



Scheme 2.6: Reaction scheme for the synthesis of polyurea (4-APhe-MMDI).

Procedure

Under nitrogen atmosphere, NMP (6.0 ml) was added to 4APhe (500 mg, 2.78 mmol) and the mixture was stirred under ice cooling. Then, 4,4'-diisocyanate-3,3'-dimethyldiphenylmethane (MMDI, 694 mg, 2.78 mmol) was added and stirred for 40 hours, and the reaction solution was added dropwise to distilled water to reprecipitate the polymer. The precipitate was filtered, washed with methanol and vacuum dried to obtain white fibril-like polyurea (4APhe-MMDI).

Yield: 1.08 g, **Yield:** 92%

Number average molecular weight M_n: 1.72 x 10^6,

Weight average molecular weight M_w: 2.21 x 10^6.

^{1}H-NMR (400 MHz, DMSO-d6): δ 2.11-2.17 (d, 6H, METHYLENE), 2.85-3.00 (d, 2H, METHYLENE), 3.70-3.75 (t, 2H, METHYLENE), 4.40-4.41 (D, 1H, METHYLENE), 6.69-6.70 (d, 1H, UREA), 6.89-7.69 (m, 10H, aromatic ring), 7.83 (s, 2H, Urethane), 8.91 (s, 1H, UREA).

13C NMR (100 MHz, DMSO-d6): δ 18.0, 37.0, 39.7, 53.9, 117.9, 121.0, 121.4, 126.3, 127.2, 127.8, 129.7, 130.3, 130.4, 135.3, 135.8, 136.0, 138.5, 152.8, 155.0, 173.7.

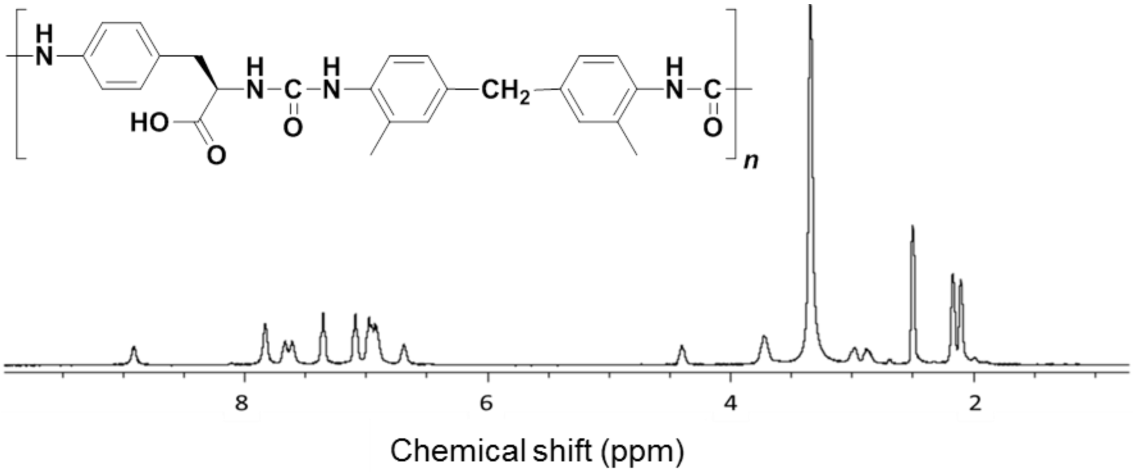


Figure 19. ^{1} H NMR spectrum of polyurea (4APhe-MMDI).

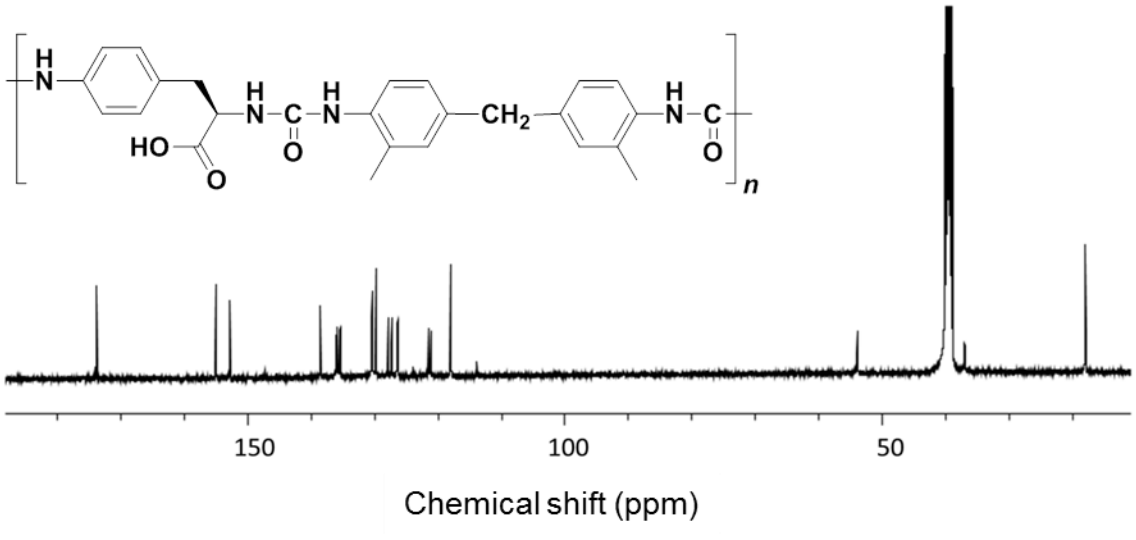


Figure 20. ^{13}C NMR spectrum of polyurea (4APhe-MMDI).

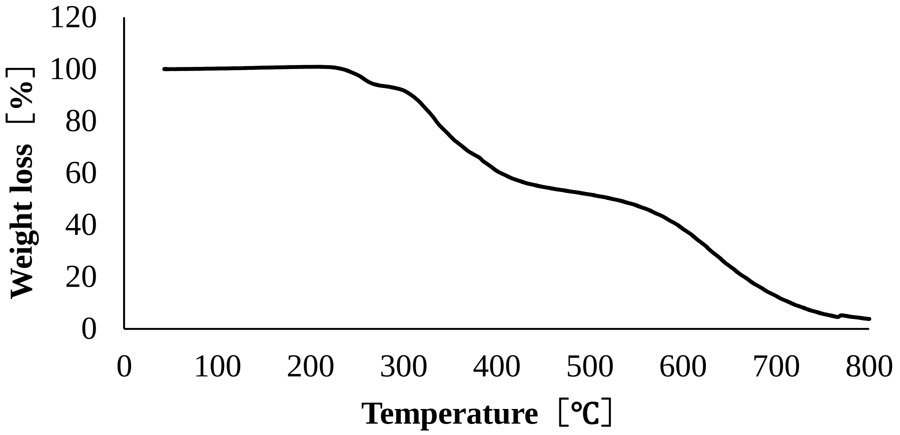
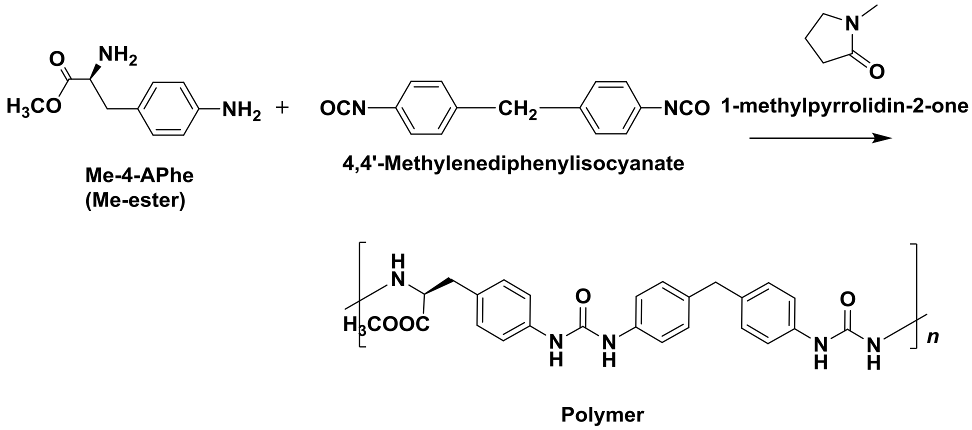


Figure 21. TGA Curve of polyurea (4APhe-MMDI).

5. Synthesis of polyurea (Me-4APhe-MDI) using 4,4-diphenylmethane diisocyanate



Scheme 2.7: Reaction scheme for the synthesis of polyurea (Me-4-APhe-MDI).

Procedure:

Under nitrogen atmosphere, NMP (3.0 ml) was added to Me-4APhe (500 mg, 2.57 mmol) and the mixture was stirred under ice cooling. Next, 4,4'-diphenylmethane diisocyanate (MDI, 644 mg, 2.57 mmol) was added and stirred for 24 hours, and the reaction solution was dropped into distilled water to reprecipitate the polymer. The precipitate was filtered, washed with methanol and vacuum dried to obtain white fibrillar polyurea (Me-4APhe-MDI).

Yield: 1.20 g, **yield:** 98%

Number average molecular weight M_n: 1.37 x 10^5,

Weight average molecular weight M_w: 3.37 x 10^5

^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.90-3.19 (d, 2H, METHYLENE), 3.65 (s, 2H, METHYLENE), 3.81(s, 3H, Methyl), 4.46-4.605(dd, 1H, METHYLENE), 6.24-6.37(d, 3H, urea), 7.10-7.36 (m, 12H, aromatic ring), 8.56-8.62 (d, 3H, urea).

^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 36.8, 39.4, 49.0, 51.9, 118.2, 118.7, 129.3, 130.0, 136.0, 138.1, 138.3, 153.1, 154.4.

Figure 22. Physical appearance of polymer (Me-4APhe-MDI).

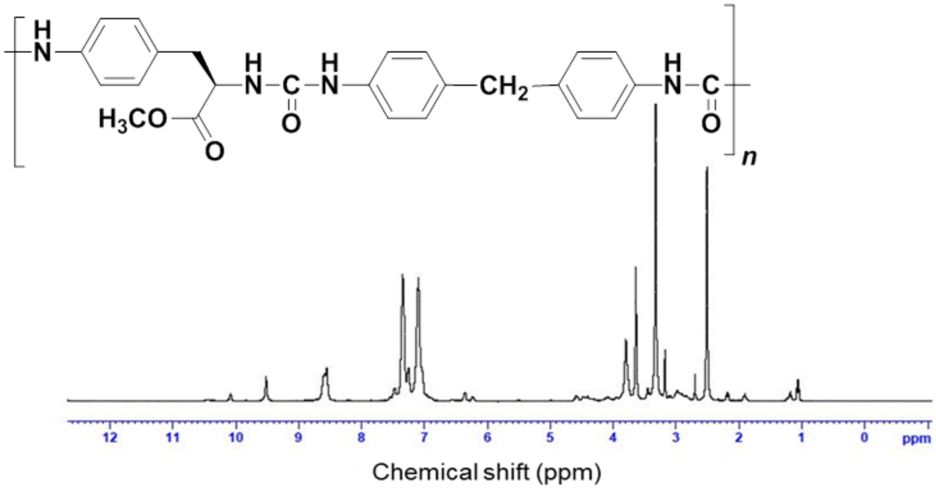


Figure 23. ^{1}H-NMR of polyurea (Me-4APhe-MDI).

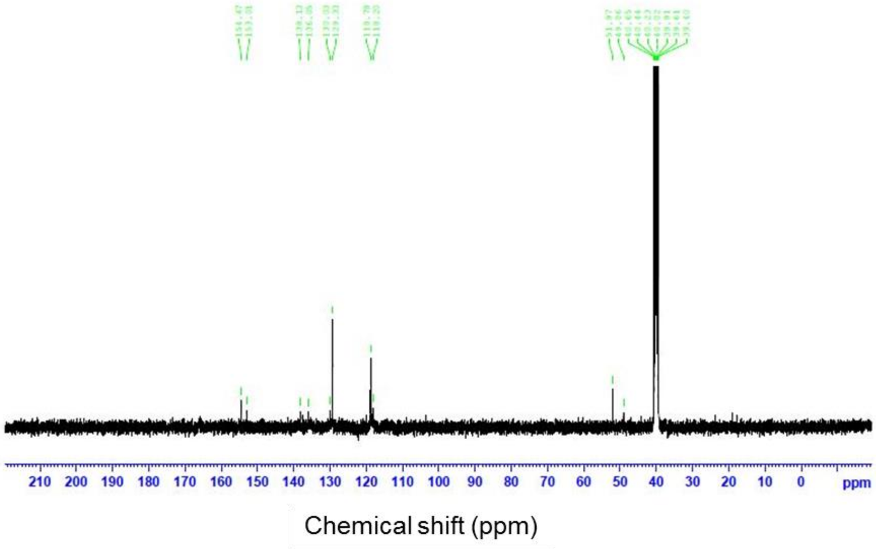
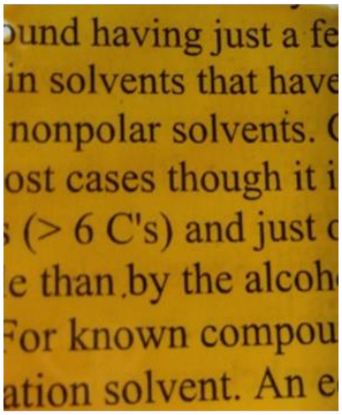


Figure 24. ^{13}C-NMR of polyurea (Me-4APhe-MDI).



ound having just a fe
in solvents that have
nonpolar solvents.
ost cases though it i
(> 6 C's) and just
e than by the alcoh
or known compou
ation solvent. An e

Figure 25. Transparent yellow-colored film of polyurea (Me-4APhe-MDI).

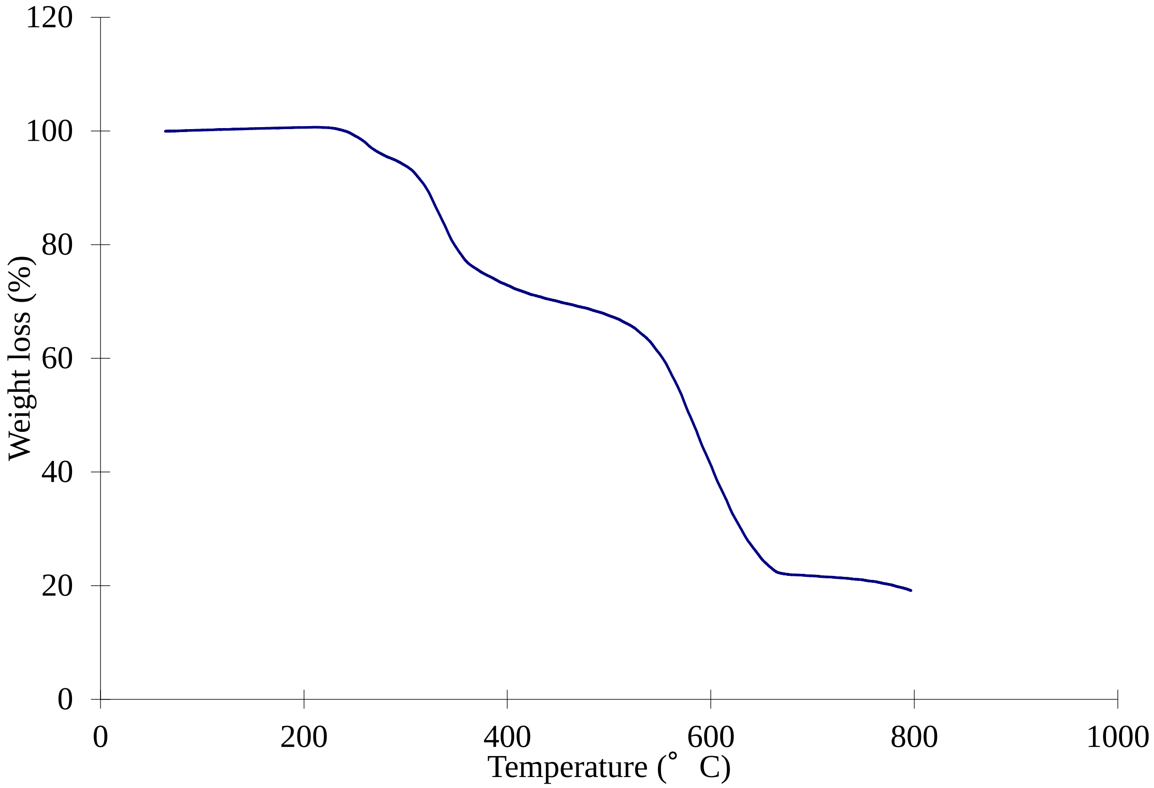


Figure 26. TGA Curve of polyurea (Me-4APhe-MDI).

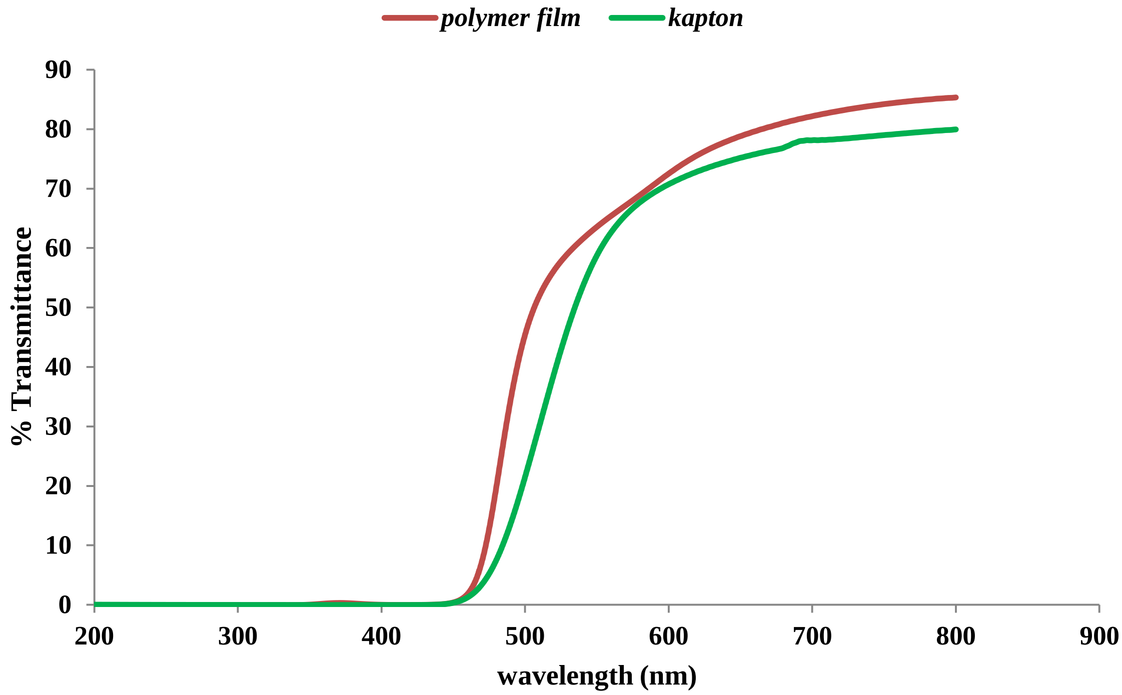


Figure 27. Transmittance of polyurea (Me-4APhe-MDI).

(*The polymer film showed more transparency than commercially available Kapton. UV cut off wavelength, λo, and transmittances at 650 nm, T650 nm, were measured by UV/vis spectrophotometer).*

Polymers	**λ_o (nm)^c**	**$T_{650\ nm}$ (%)^c**
Kapton	435.5 nm	75.1914
PU	348 nm	78.8238

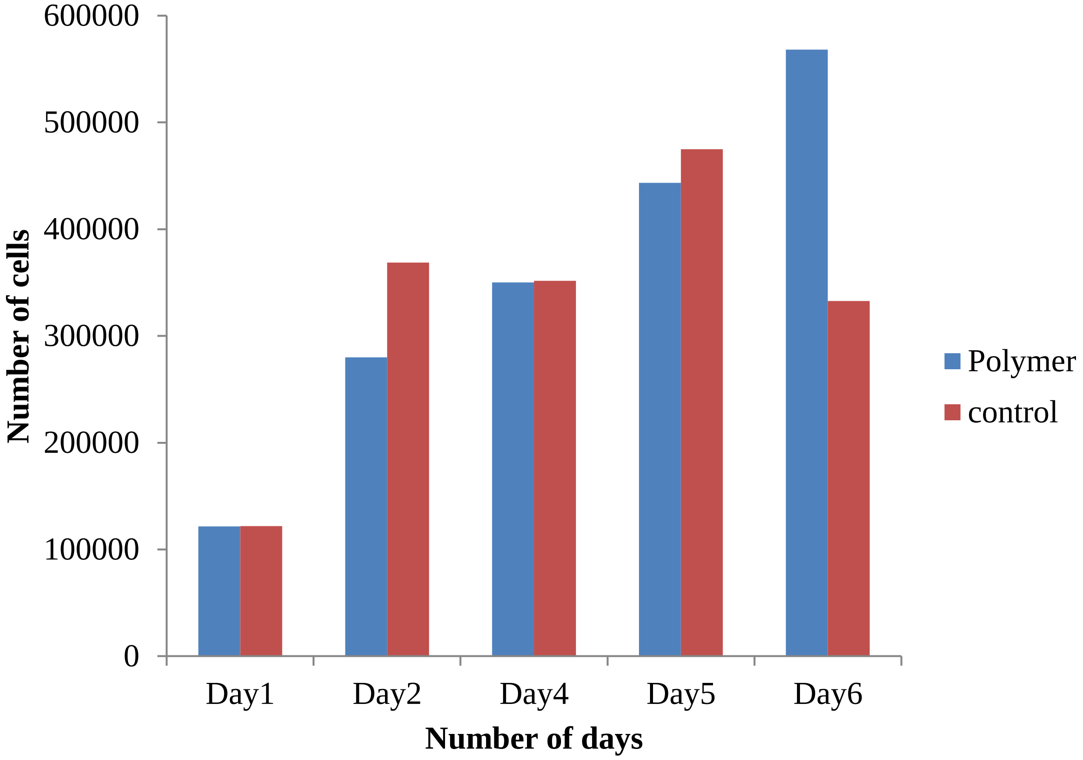
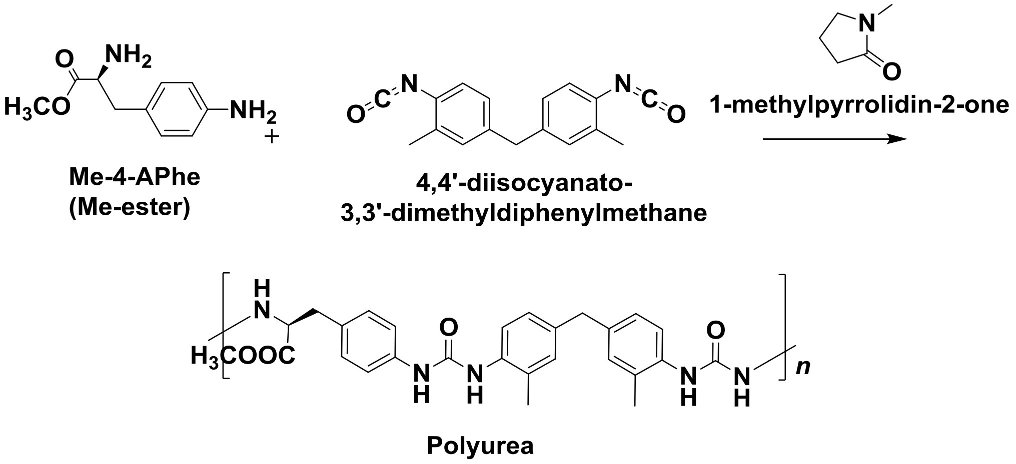


Figure 28. Cell culturing experiment showing time dependence of cell numbers of L929 fibroblasts adhered on polymer film (*The polymer film showed good cytocompability*).

6. Synthesis of polyurea (Me-4APhe-MMDI) using 4,4-diphenylmethane diisocyanate.



Scheme 2.8: Reaction scheme for the synthesis of polyurea (Me-4-APhe-MMDI).

Procedure:

Under nitrogen atmosphere, NMP (2.0 ml) was added to 4APhe (200 mg, 1.03 mmol) and the mixture was stirred under ice cooling. Then, 4,4'-diisocyanate-3,3'-dimethyldiphenylmethane (MMDI, 280 mg, 1.03 mmol) was added and stirred for 40 hours, and the reaction solution was added dropwise to distilled water to reprecipitate the polymer. The precipitate was filtered, washed with methanol and vacuum dried to obtain white fibril-like polyurea (Me-4APhe-MMDI).

Yield: 467 mg, **Yield:** 97%

Number average molecular weight M_n: 1.58×10^5,

Weight average molecular weight M_w: 3.76×10^5.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.09(d, 3H, Methyl), 2.16(d, 3H, Methyl), 2.90-3.19 (d, 2H, METHYLENE), 3.65 (s, 2H, METHYLENE), 3.81(s, 3H, Methyl), 4.46-4.605(dd, 1H, METHYLENE), 6.24-6.37(d, 3H, urea), 7.10-7.36 (m, 12H, aromatic ring), 8.56-8.62 (d, 3H, urea).

^{13}C NMR (100 MHz, DMSO-d_6): δ 17.2, 17.8, 36.8, 39.4, 49.0, 51.9, 118.2, 118.7, 129.3, 130.0, 136.0, 138.1, 138.3, 153.1, 154.4.

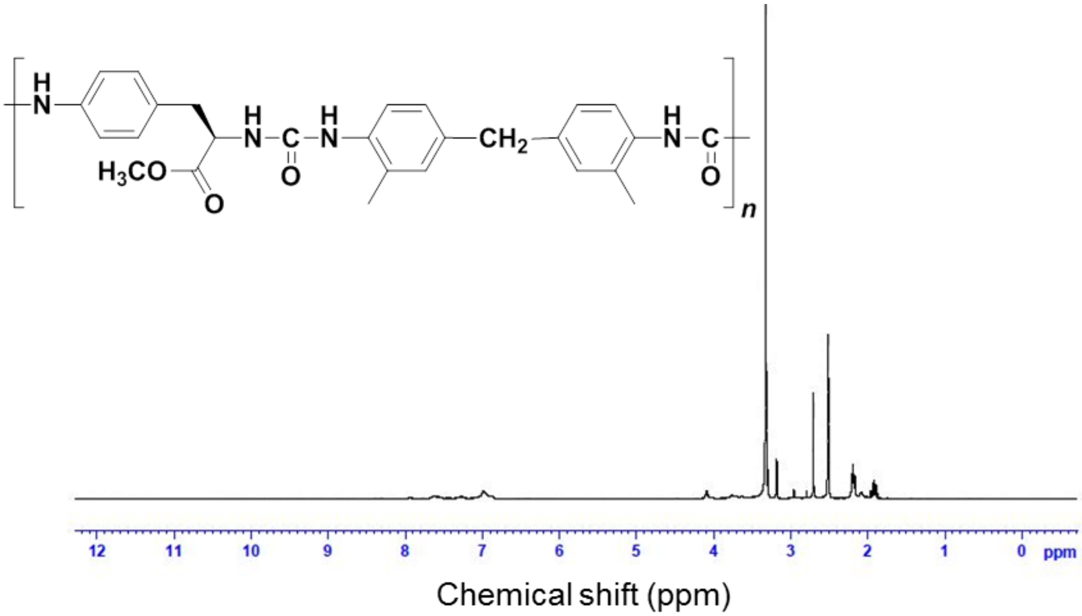


Figure 29. ^{1}H-NMR of polyurea (Me-4APhe-MMDI).

Figure 30. Physical appearance of polymer (Me-4APhe-MMDI).

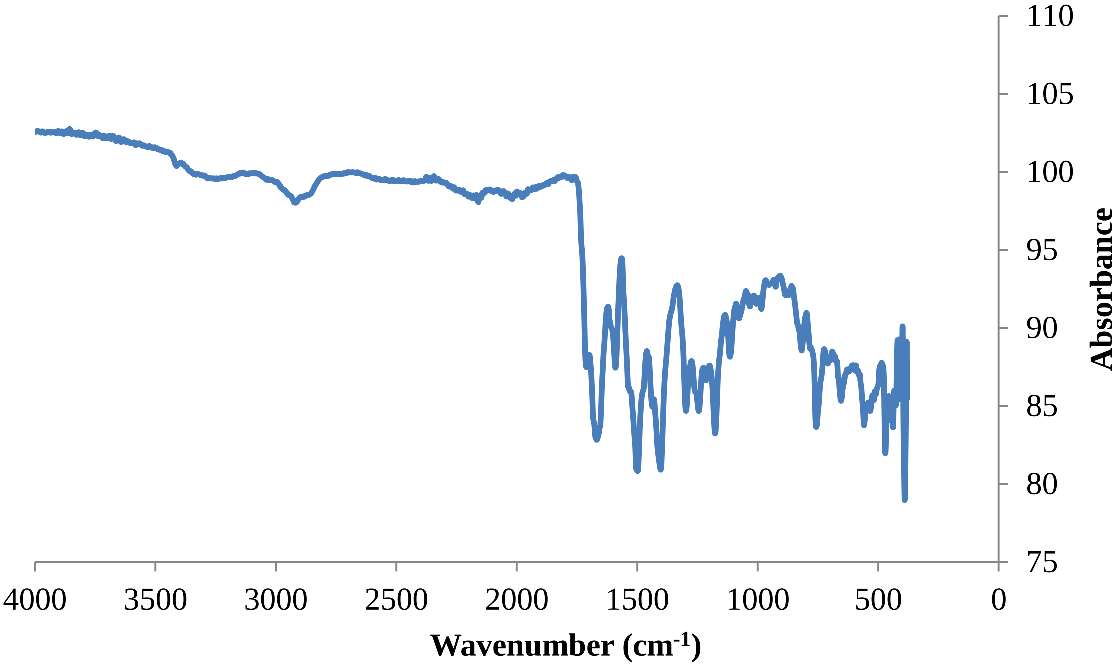


Figure 31. IR spectra of polyurea (Me-4APhe-MMDI).

(IR bends at 1620-1280 cm^{-1} *are of urea linkage while other aliphatic and aromatic segments appear at their expected positions in IR spectra).*

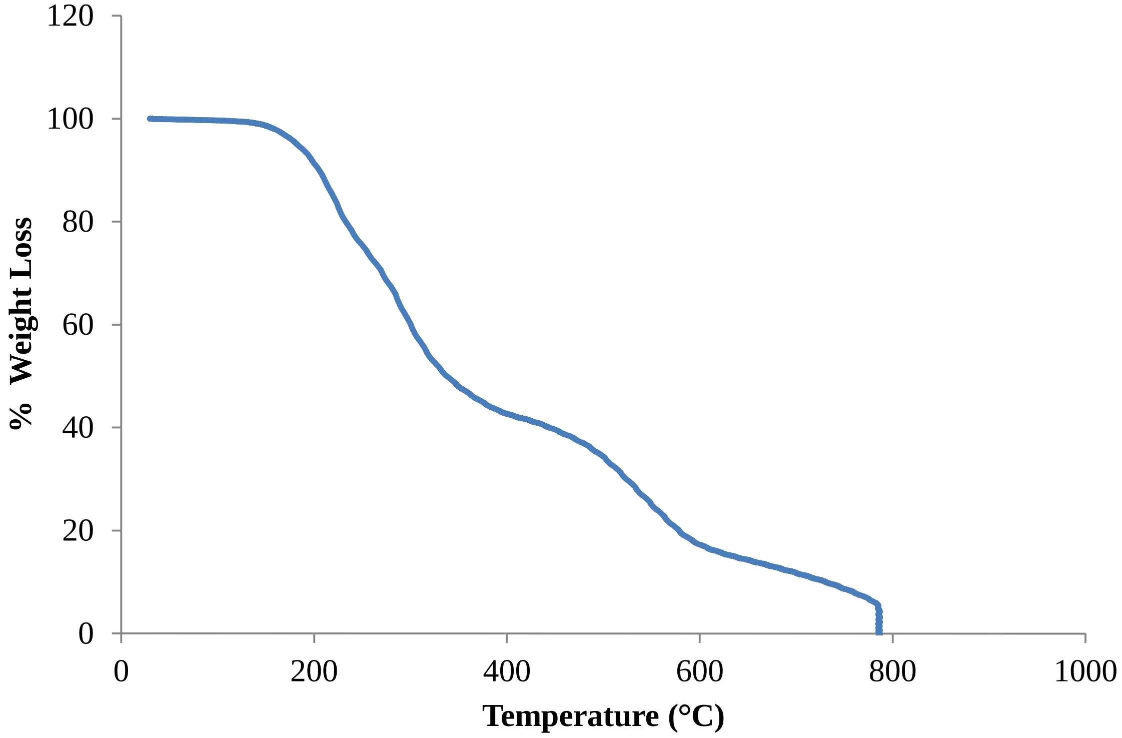


Figure 32. TGA curve of polyurea (Me-4APhe-MMDI).

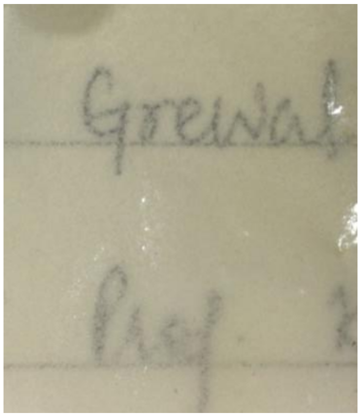


Figure 33. Film of polyurea (Me-4APhe-MMDI) in DMF.

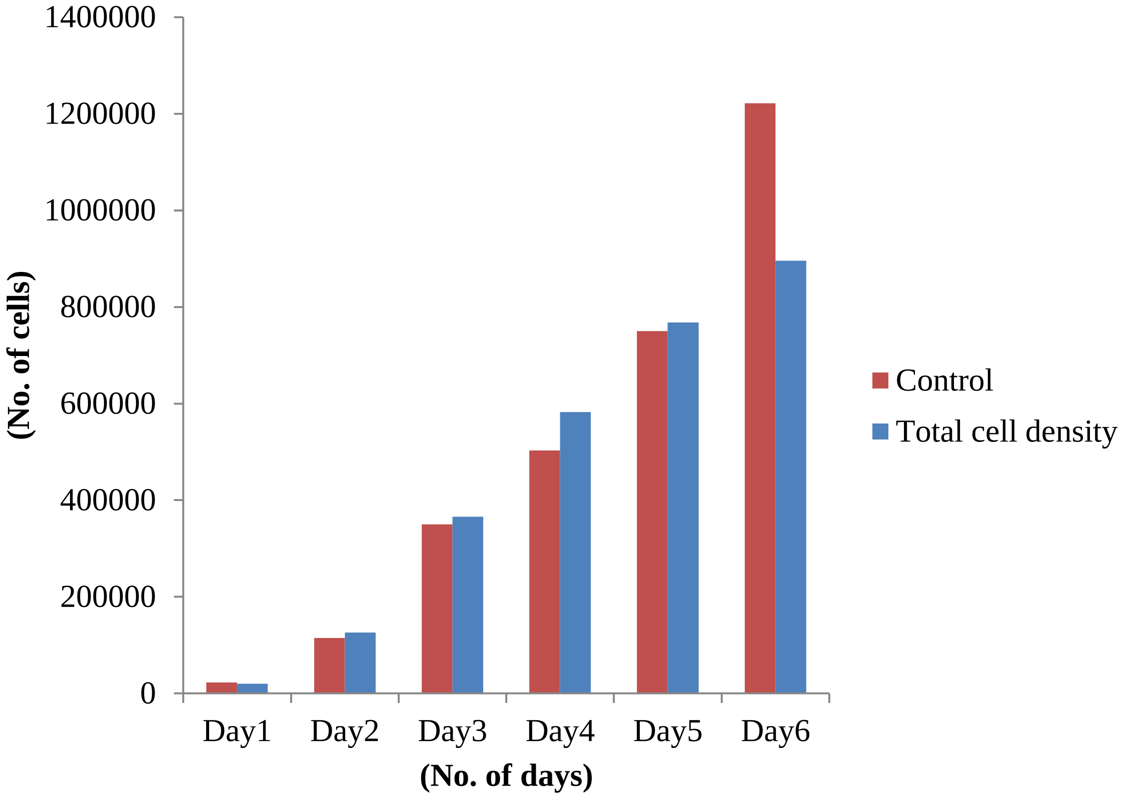
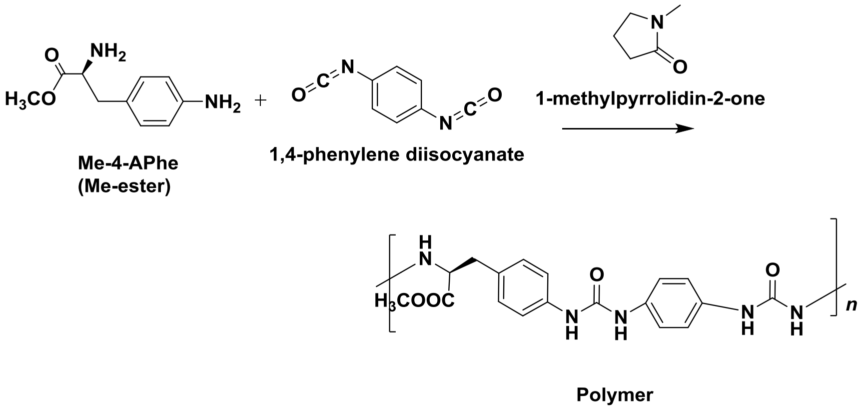


Figure 34: Cell culturing experiment showing time dependence of cell numbers of L929 fibroblasts adhered on polyurea (Me-4APhe-MMDI) film.

The polymer film showed good cytocompability.

7. Synthesis of polyurea (Me-4APhe-PDI) using 1,4-Phenylene diisocyanate (PDI).



Scheme 2.9: Reaction scheme for the synthesis of polyurea (Me-4-APhe-1,4PDI).

Procedure:

Under nitrogen atmosphere, NMP (1.0 ml) was added to 4APhe (100 mg, 0.51 mmol) and the mixture was stirred under ice cooling. Then, 1,4-phenylene diisocyanate (1,4-PDI, 80 mg, 0.51 mmol) was added and stirred for 30 hours, and the reaction solution was added dropwise to distilled water to reprecipitate the polymer. The precipitate was filtered, washed with methanol and vacuum dried to obtain white fibril-like polyurea (4APhe-PDI).

Physical appearance

Figure 35. Physical appearance of polyurea (Me-4APhe-1,4PDI).

Yield: 150 mg, **Yield:** 85 %

Number average molecular weight M_n: 2.22×10^5,

Weight average molecular weight M_w: 2.49×10^5.

^{1}H-NMR (400 MHz, DMSO-d6): δ 2.90-2.97 (d, 2H, methylene), 3.05-3.20 (d, 2H, methylene), 4.39 (s, 1H, methylene), 6.25-6.41 (t, 1H, urea), 7.15-7.57 (aromatic ring), 8.72 (t, 3H, urea).

^{13}C NMR (100 MHz, DMSO-d6): δ 32.2, 37.0, 52.0, 65.7, 118.5, 129.4, 130.0, 134.2, 138.5, 140.60, 152.7, 154.8, 173.8.

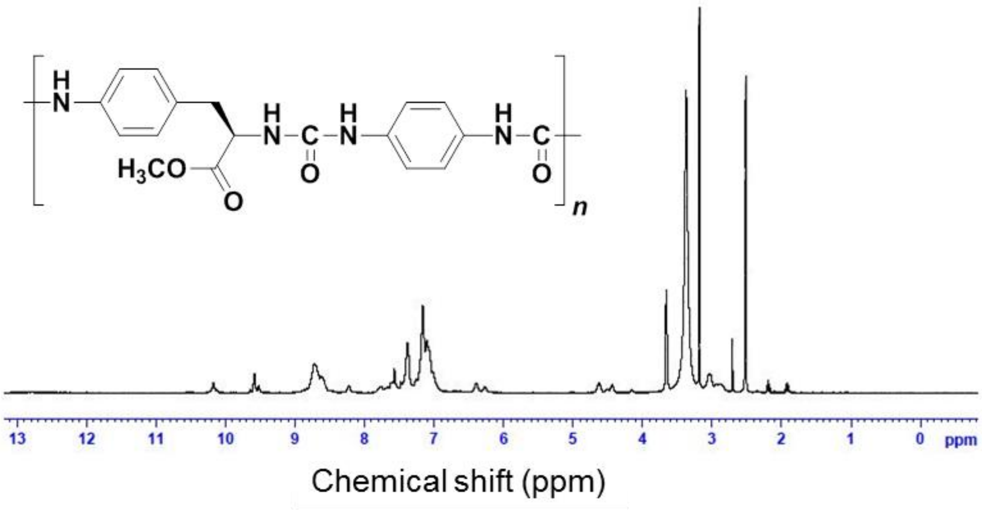


Figure 36. ^{1}H-NMR of polymer (Me-4APhe-1,4PDI).

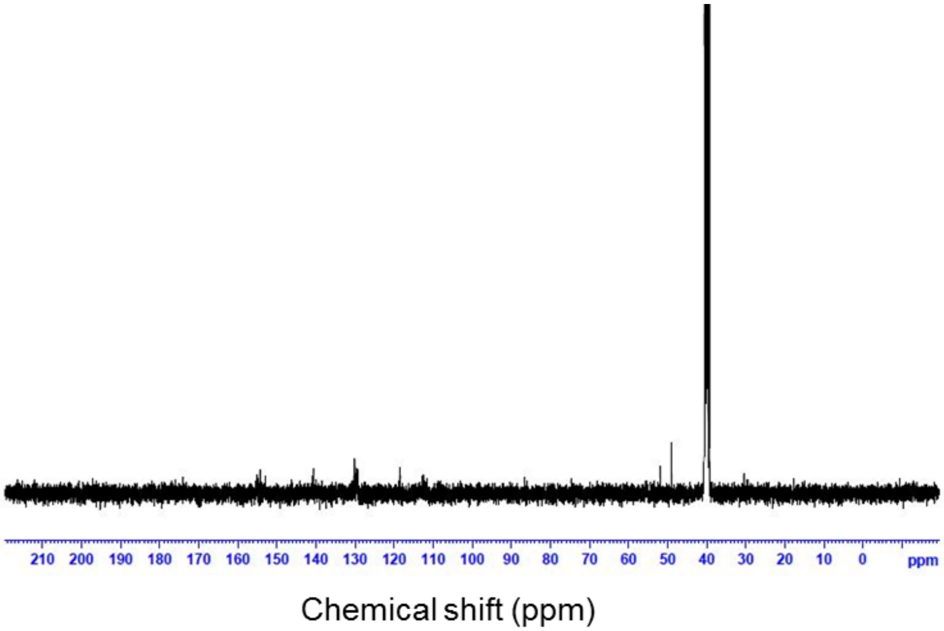


Figure 37. ^{13}C-NMR of polyurea (Me-4APhe-1,4PDI).

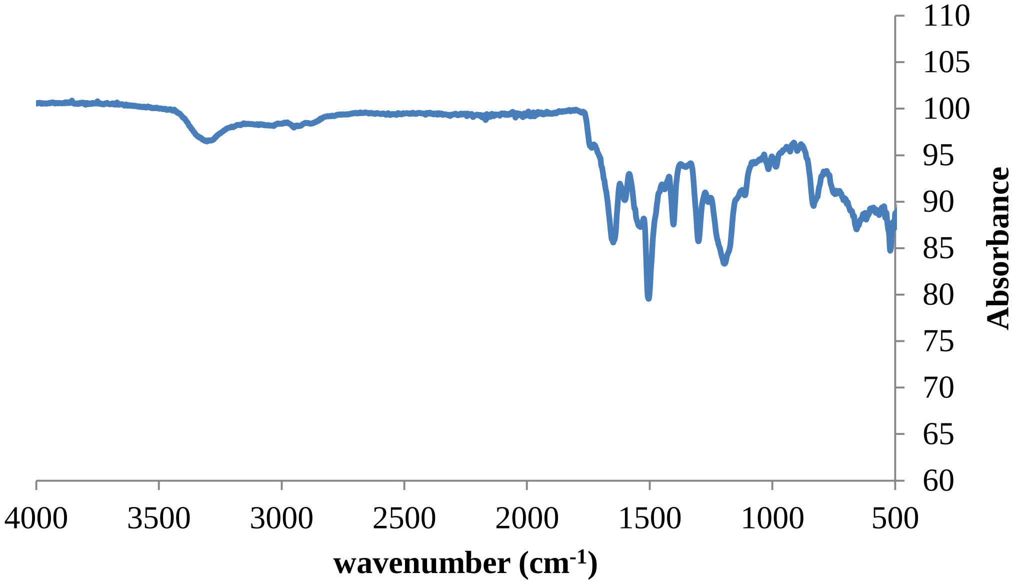


Figure 38. IR spectra of polyurea (Me-4APhe-1,4PDI).

Broad absorption bands in the range of 3314 cm^{-1} *(N-H stretching), carbonyl peak at 1740 cm*$^{-1}$ *(C=O stretching, ester) and 1650 cm*$^{-1}$ *(C=O stretching, urea), and aromatic peaks at 1518 cm*$^{-1}$ *(C-H stretching).*

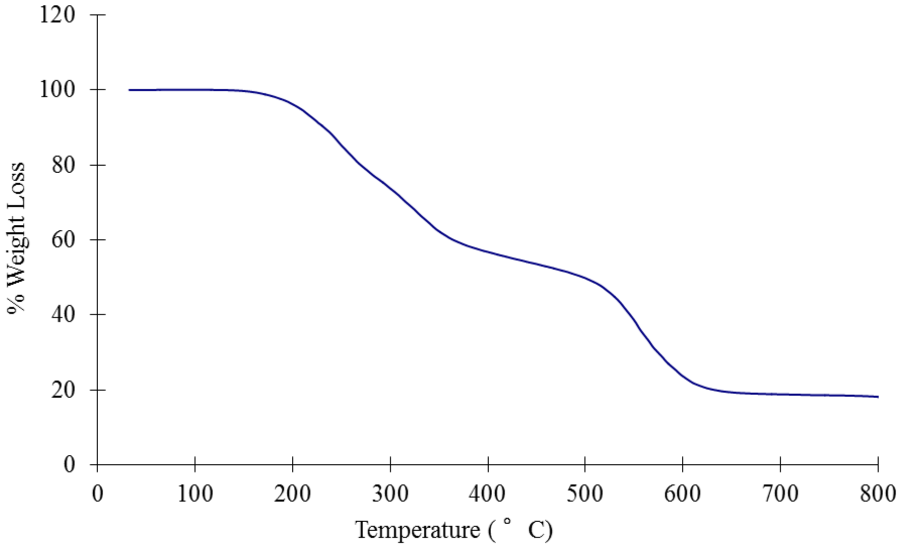
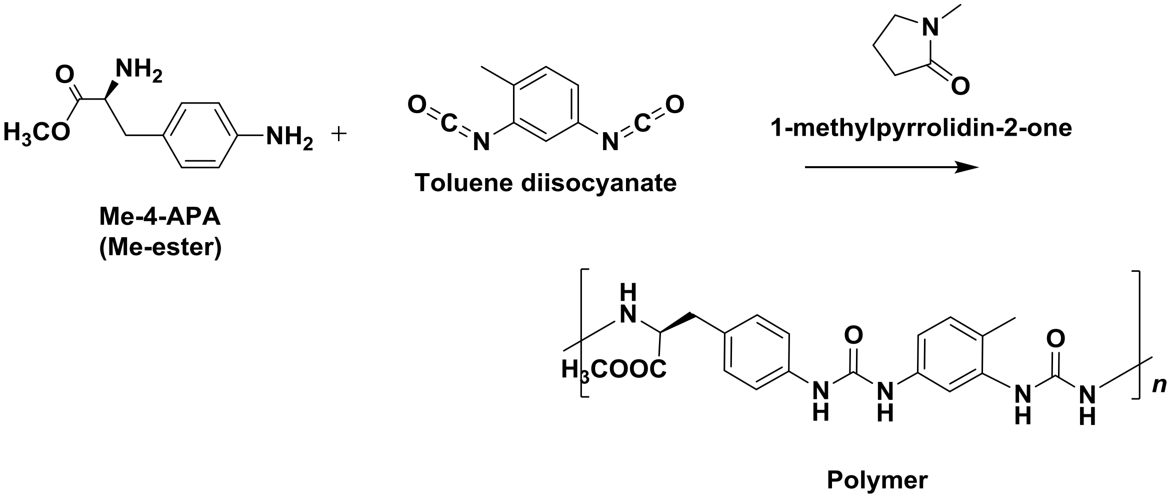


Figure 39. TGA of polyurea (Me-4APhe-1,4PDI).

8. Synthesis of polyurea (Me-4APhe-TDI) using Toluene diisocyanate (TDI).



Scheme 2.10: Reaction scheme for the synthesis of polyurea (Me-4-APhe-TDI).

Procedure:

Under nitrogen atmosphere, NMP (1.0 ml) was added to 4APhe (100 mg, 0.51 mmol) and the mixture was stirred under ice cooling. Then, toluene diisocyanate (TDI, 80 mg, 0.51 mmol) was added and stirred for 30 hours, and the reaction solution was added dropwise to distilled water to re-precipitate the polymer. The precipitate was filtered, washed with methanol and vacuum dried to obtain white fibril-like polyurea (4APhe-TDI).

Yield: 160 mg, **% Yield:** 89 %

Figure 40. Physical appearance of polyurea (Me-4Aphe-TDI).

Number average molecular weight M_n: 8.80 x 10^4,

Weight average molecular weight M_w: 1.7 x 10^5

PDI: 1.925

^{1}H-NMR (400 MHz, DMSO-d6): δ 2.90-2.97 (d, 2H, METHYLENE), 4.39 (s, 1H, METHYLENE), 6.18-6.21 (t, 1H, UREA), 7.08-7.34, Aromatic ring), 8.47-8.57 (t, 3H, urea).

^{13}C NMR (100 MHz, DMSO-d6): δ 36.8, 53.7, 118.1, 118.9, 129.6, 130.2, 134.2, 138.5, 152.7, 154.8, 173.8.

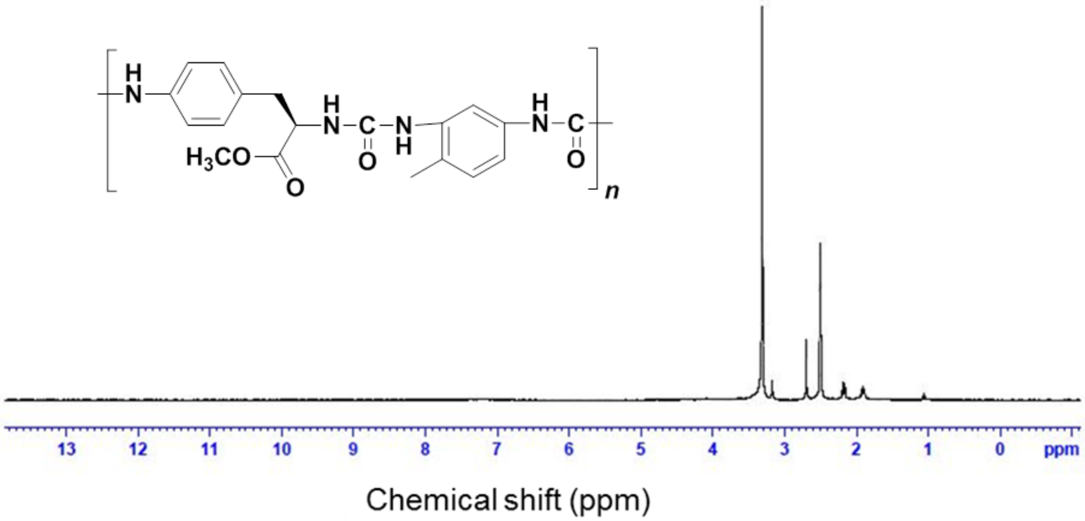


Figure 41: ^{1}H-NMR of polymer (Me-4APhe-TDI).

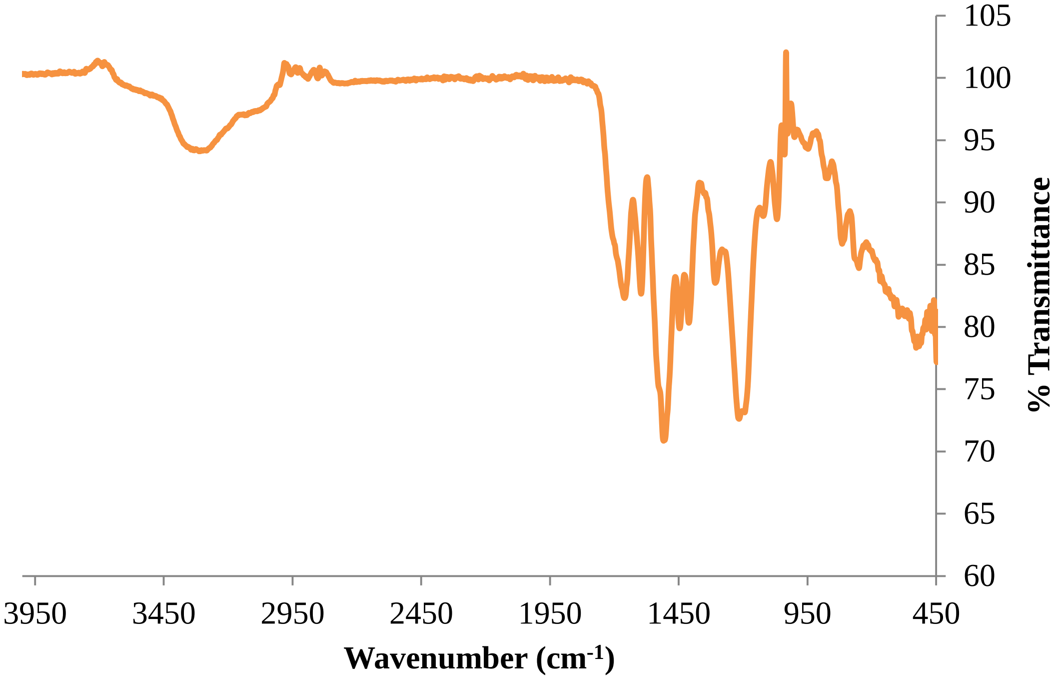


Figure 42. IR spectroscopy of polymer (Me-4APhe-TDI).

IR bends at 1620-1280 cm^{-1} may be because of urea linkage while other aliphatic and aromatic segments appear at their expected positions in IR spectra.

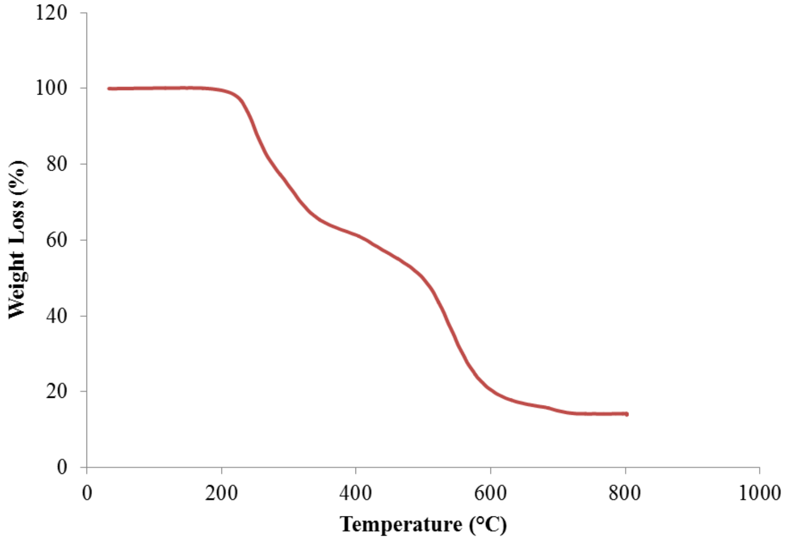
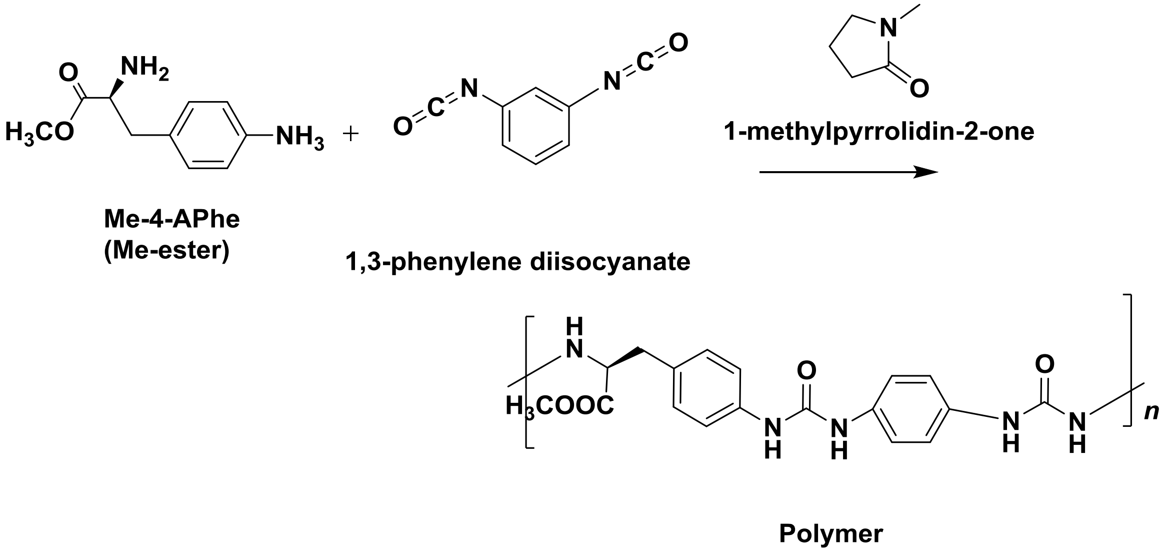


Figure 43. TGA pattern of polyurea (Me-4APhe-TDI).

9. Synthesis of polyurea (Me-4APhe-PDI) using 1,3-Phenylene diisocyanate (PDI).



Scheme 2.11: Reaction scheme for the synthesis of polyurea (Me-4-APhe-1,3PDI).

Procedure:

Under nitrogen atmosphere, NMP (1.0 ml) was added to 4APhe (100 mg, 0.51 mmol) and the mixture was stirred under ice cooling. Then, 1,3-phenylene diisocyanate (1,3-PDI, 80 mg, 0.51 mmol) was added and stirred for 30 hours, and the reaction solution was added dropwise to distilled water to reprecipitate the polymer. The precipitate was filtered, washed with methanol and vacuum dried to obtain white fibril-like polyurea (4APhe-PDI).

Yield: 150 mg, **% Yield:** 85 %

Figure 44. Physical appearance of polyurea (Me-4APhe-1,3PDI)

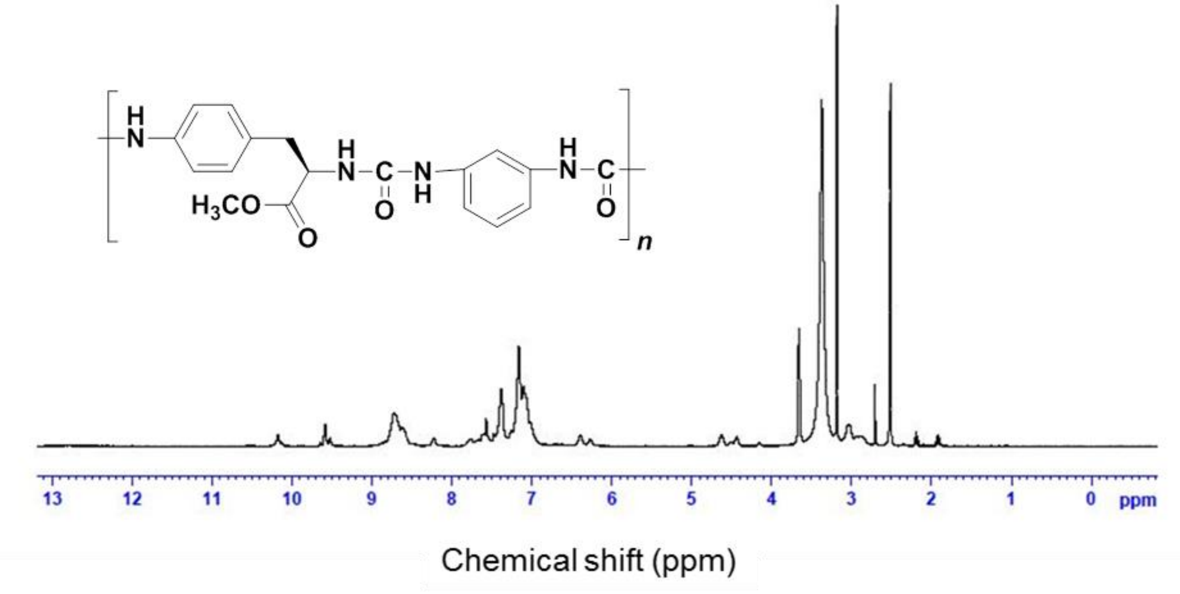


Figure 45: ^{1}H-NMR of polymer (Me-4APhe-1,3-PDI).

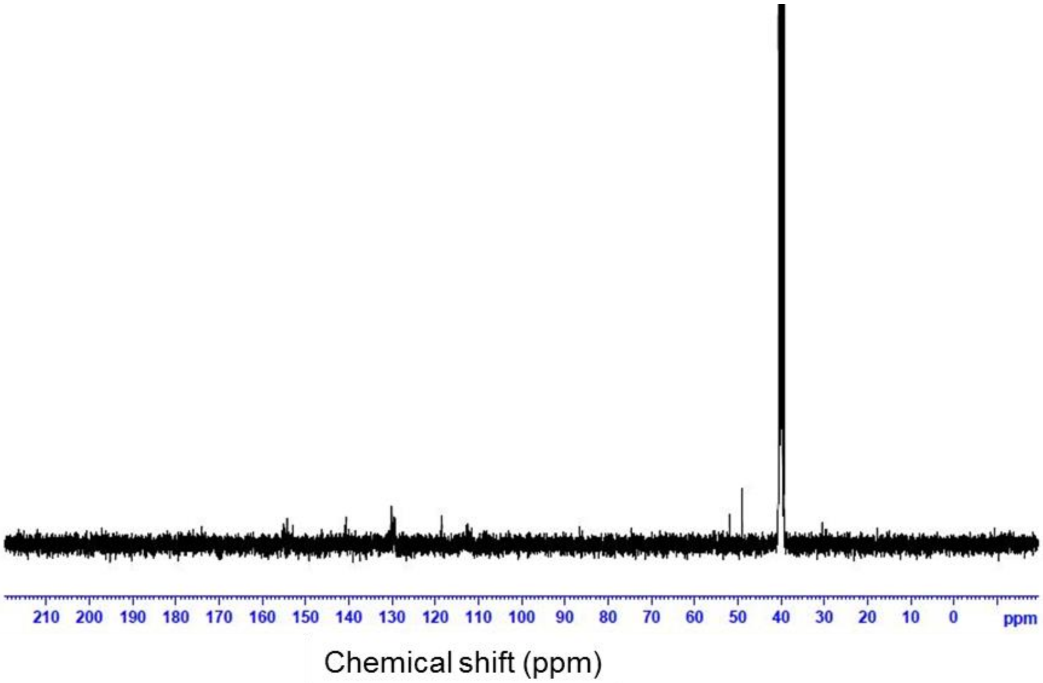


Figure 46. ^{13}C-NMR of polyurea (Me-4APhe-1,3-PDI).

Number average molecular weight M_n: 2.52 x 10^5,

Weight average molecular weight M_w: 6.46 x 10^5.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.90-2.97 (d, 2H, METHYLENE), 3.05-3.20 (d, 2H, METHYLENE), 4.39 (s, 1H, METHYLENE), 6.25-6.41 (t, 1H, UREA), 7.15-7.57 (Aromatic ring), 8.72 (t, 3H, Urea)

^{13}C NMR (100 MHz, DMSO-d_6): δ 32.2, 37.0, 52.0, 65.7, 118.5, 129.4, 130.0, 134.2, 138.5, 140.60, 152.7, 154.8, 173.8.

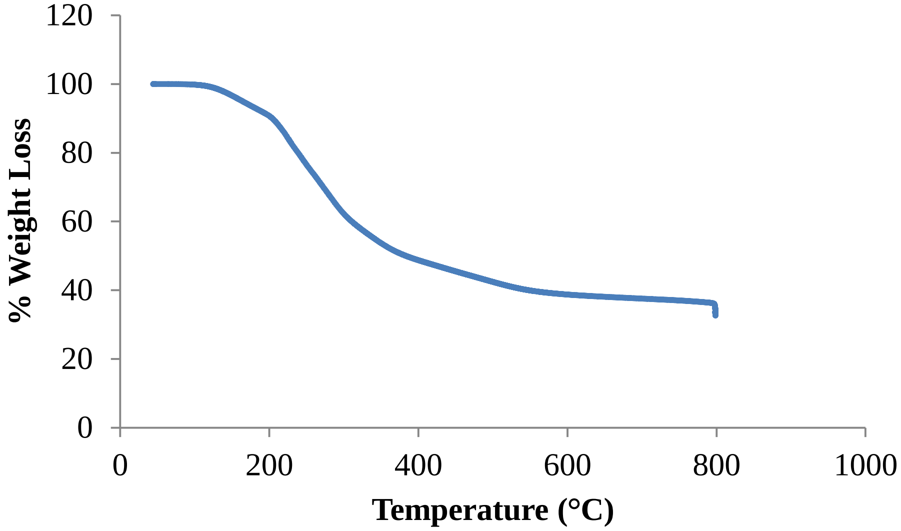


Figure 47. TGA curve of polyurea(Me-4-APhe-1,3-PDI).

2.3 Discussion

4APhe was stored in hydrochloride salt state to prevent oxidation of amine groups after whitebio-production. In order to carry out the efficient polymerization, 4APhe·2HCl salt was neutralized by addition of appropriate amount of NaOH in water, to be ready to polymerize. On the other hand, the methyl ester of 4APhe was produced for comparison in physical properties of the resulting polymers. The monomer structures were confirmed using ^{1}H NMR, ^{13}C NMR, FT-ICR-MS, and FT-IR.

A series of PUs were prepared by polyadditions of 4APhe or its methyl ester with stoichiometric amounts of aromatic diisocyantes such as MDI, MMDI, 1,3-PDI, and 1,4-PDI (Scheme 3). In this paper, PUs with side groups of carboxylic acid and carboxylate methyl ester are abbreviated as PU-*X*a and PU-*X*b, respectively, where X = 1, 2, 3, and 4 are MDI, MMDI, 1,4-PDI, and 1,3-PDI, respectively. The ^{1}H NMR spectra of these PUs indicate the proton signals for aromatic region, ureas, methyl ester, β-methyne, α-methylene, around 7.0-7.5, 8.5-8.7 and 6.20-6.26, 3.2, 2.80-2.99 and 4.40 ppm, respectively. Carboxylic acid proton of PU-*X*a appeared around 12.2 ppm as a broad signal. The weight average molecular weight (M_w), the number average molecular weight (M_n), and the molecular weight distribution (PDI) of the synthesized PUs were evaluated by GPC (Table 2). PU-*X*a with free carboxylic acid showed high M_n and M_w values ranging 1.25-1.72 × 10^5 and 1.63-2.80 × 10^5, respectively, and then PDI ranged 1.34-1.63. However, PU-*X*b with methyl ester side group showed M_n ranging 1.37-2.57 × 10^5 and M_w ranging 3.37- 6.46 × 10^5. Overall, PUs prepared here showed molecular weights high enough to evaluate the thermal and mechanical properties.

The solubility of all the prepared PUs was tested at room temperature in different solvents: (1) polar protic solvents such as distilled water, methanol, ethanol, and concentrated sulfuric acid, (2) polar aprotic solvents such as acetone, acetonitrile, ethyl acetate, DMF, NMP, DMAc, DMSO and THF, and (3) non-polar solvents such as hexane, benzene, toluene, chloroform, diethyl ether and dichloromethane (DCM). The results are summarized in Table 4. PU-*X*a was readily soluble while PU-*X*b became soluble after sometime. The solubility in some volatile solvents imparted PUs the processability into films as shown in film picture of Figure 48b processed from the fibrils of Figure 48a. All the PUs except for PU-3b and PU-4b were able to process into films. On the other hand, the insolubility of PUs in most of the common solvents proposed their possibility of chemical resistance, low permeability, and water proof nature.

Table2. Summary of PUs syntheses using 4APhe and 4APhe methyl ester.

polymers	diisocyanates	yield (%)	M_n [a] ($\times10^5$)	M_w [a] ($\times10^5$)	PDI [a]	film property
PU-1a	MDI	94	1.72	2.80	1.63	ductile
PU-2a	MMDI	92	1.32	2.08	1.57	ductile
PU-3a	1,3-PDI	99	1.71	1.63	1.52	ductile
PU-4a	1,4-PDI	72	1.25	1.68	1.34	ductile
PU-1b	MDI	98	1.37	3.37	2.45	ductile
PU-2b	MMDI	97	1.58	3.76	2.38	ductile
PU-3b	1,3-PDI	84	2.52	6.46	2.15	brittle
PU-4b	1,4-PDI	80	2.57	6.16	2.39	brittle

a) Molecular weight and its distribution was measured by gel permeation chromatography using external pullulan standards in dimethylformamide as eluent at a flow rate of 0.5 ml min^{-1} using the RI signal detector.

The thermal degradation of all the synthesized PUs was analyzed by thermogravimetric analysis (representative curves are along with the polymers data) in nitrogen atmosphere at a heating rate of 5 °C min^{-1}, and the values of 5 % and 10 % weight-loss temperatures (T_{d5} and T_{d10}) were determined, which were summarized in Table 3. TGA analyses were made up to 800 °C to evaluate the residual char amount to be nearly 5 % or less in all PUs prepared here, indicating considerable pyrolytic degradation. The T_{d5} values lie above 250 °C for most of the PU-*X*a, while T_{d10} ranged between 263-317 °C which are much higher than conventional bioplastics such as poly(lactic acid)s (PLA, ca. 300 ^{o}C) (46) and polyhydroxybutyrate (PHB, 260 ^{o}C) (47). On the other hand, these weight loss temperatures are lower than those of polyesters containing aromatics such as poly(ethylene terephthalate) (PET, ca. 430 ^{o}C) (48) presumably due to lower thermal degradation resistance of urea linkage than esters. However the T_{d10} values of PU-1a and PU-1b are higher than MDI-derived polyureas from 4,4'-diaminotruxillic acid we reported previously (T_{d10} 270-280 ^{o}C) (27). This may be attributed to barrier effects of sterically-bulky carboxylic group and its derivative on protecting contiguous urea linkage connecting to 4APhe component. Among isocyanate used here, MDI has the best combination with 4APhe in terms of thermal degradation resistance. Overall almost all of PU-*X*a have higher thermal degradation temperature than PU-*X*b if the values were compared in PUs having the corresponding isocyanate components. The results can be discussed owing to interchain hydrogen bonding of carboxylic acid. The representation of intermolecular hydrogen bonding between polyureas is illustrated in Figure 49, showing a variety of hydrogen bonding of carboxylic acids with ureas or of themselves. Overall, PUs showed good thermal stability until 200 °C which is high compared to conventional polyureas (aliphatic) or polyurea spray elastomer (Spandex) (48).

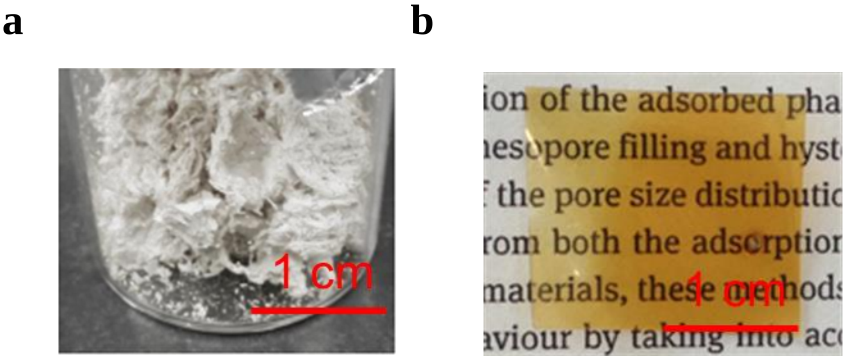


Figure 48. Representative photos of PU materials prepared from 4-aminophenylalanine with diisocyanate (PU-1b). a) as-prepared sample, b) cast film.

The T_g values of PUs determined by DSC are summarized in Table 4. T_g values of PU-*X*a ranged from 148 to 190 °C, which are higher than those of PU-*X*b presumably due to the abovementioned hydrogen bonding effects of carboxylic acid. PU-3a and PU-4a showed higher T_g values than PU-1a and PU-2a, which can be due to the high chain rigidity of PU-3a and PU-4a originated from direct urea connection to phenylene group without any connecting methylene. There can be arose similar discussion about T_g comparison of PU-3b and PU-4b with PU-1b and PU-2b. On the other hand, T_g values of PU-*X*a are higher than those of conventional polyesters such as PLA, PHA and PET (45-48) and even comparison with polycarbonate T_g some of PUs prepared here had higher values, which have a good advantage for applications in plastic industry for temperature tolerant.

Table 3. Thermal and mechanical properties of bio-based PUs from 4APhe and 4APhe methyl ester.

Polymers	**T_{d5} ^{a} (°C)**	**T_{d10} ^{a} (°C)**	**T_g ^{b} (°C)**	**σ ^{c} (MPa)**	**E_{init} ^{c} (GPa)**	**ε ^{c} (%)**	**U ^{c} (MJ/m^3)**
PU-1a	291	317	155	147 ± 16 (54 ± 14)	5.5 ± 1.8 (1.3 ± 0.1)	11 ± 7.2 (5.4 ± 1.3)	14 ± 10 (1.7 ± 0.3)
PU-2a	263	308	148	145 ± 15 (43 ± 9.2)	7.9 ± 3.1 (1.2±0.4)	7.8 ± 1.5 (7.4 ± 2.3)	8.9 ± 2.2 (2.2 ± 0.3)
PU-3a	248	263	190	59 ± 13	5.8 ± 1.2	1.0 ± 0.3	0.29 ± 0.12
PU-4a	267	297	190	156 ± 15	6.9 ± 2.5	8.2 ± 2.7	10 ± 2.3
PU-1b	288	318	120	(31 ± 15)	(3.1 ± 0.4)	(2.2 ± 1.2)	(1.1 ± 0.05)
PU-2b	182	203	100	(10 ± 2)	(1.8 ± 0.4)	(5.0 ± 0.4)	(0.46 ± 0.05)
PU-3b	180	265	165	ND	ND	ND	ND
PU-4b	200	318	140	ND	ND	ND	ND
PA 11 ^{d}	-	-	29	67	1.3	-	-
PET ^{d}	-	-	80	80	3.0	70	-
PLA ^{d}	-	-	60	80	3.5	6.0	-
PHA ^{d}	-	-	2	25	1.5	10	-
PC ^{d}	-	-	150	62	1.9	200	-
PMMA ^{d}	-	-	121	60	2.3	3.1	-

a) T_{d5} and T_{d10} refer to temperatures at 5 % and 10 % weight loss, respectively, measured using TGA at a heating rate of 5 °C/min under N_2. b) T_g refers to glass transition temperature, measured by DSC at a heating rate of 10 °C/min under N_2 atmosphere. c) Tensile strength at break, σ, Young's modulus, E_{init}, elongation at break, ε, and strain energy density, U, were estimated from stress-strain curves recorded using tensiometer at room temperature. ND means not determined due to brittleness. Values in parentheses are determined using film specimens. d) PA11, F-PI, PET, PHA, PLA, PC, and PMMA refer to poly(11-aminoundecanoic acid), fluorinated polyimide (from hexafluoroisopropylidene diphthalic anhydrides and 2,5-diamino-2,5-dideoxy-1,4: 3,6-dianhydrosorbitol), poly(ethylene terephthalate), polyhydroxyalkanoates, poly(lactic acid), poly(bisphenol A carbonate), and poly(methyl methacrylate), respectively (45, 46).

Table 4. Solubility chart for different PUs in different solvents.

Polymer → **Solvent**	**PU-1a**	**PU-2a**	**PU-3a**	**PU-4a**	**PU-1b**	**PU-2b**	**PU-2c**	**PU-2d**	**PU-2e**
Water	-	-	-	-	-	-	-	-	-
Methanol	-	-	-	-	-	-	-	-	-
Ethanol	-	-	-	-	-	-	-	-	-
Acetonitrile	-	-	-	-	-	-	-	-	-
tetrahydrofuran	-	-	-	-	-	-	-	-	-
Diethylether	-	-	-	-	-	-	-	-	-
DMF	+	+	+	+	+	+	+	+	+
DMAc	+	+	+	+	+	+	+	+	+
NMP	+	+	+	+	+	+	+	+	+
DMSO	±	±	±	±	±	±	±	±	±
Ethyl acetate	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-	-
Hexane	-	-	-	-	-	-	-	-	-
Conc. H_2SO_4	+	+	+	+	+	+	+	+	+

+:Soluble at room temperature, -: Insoluble , ±: partially soluble.

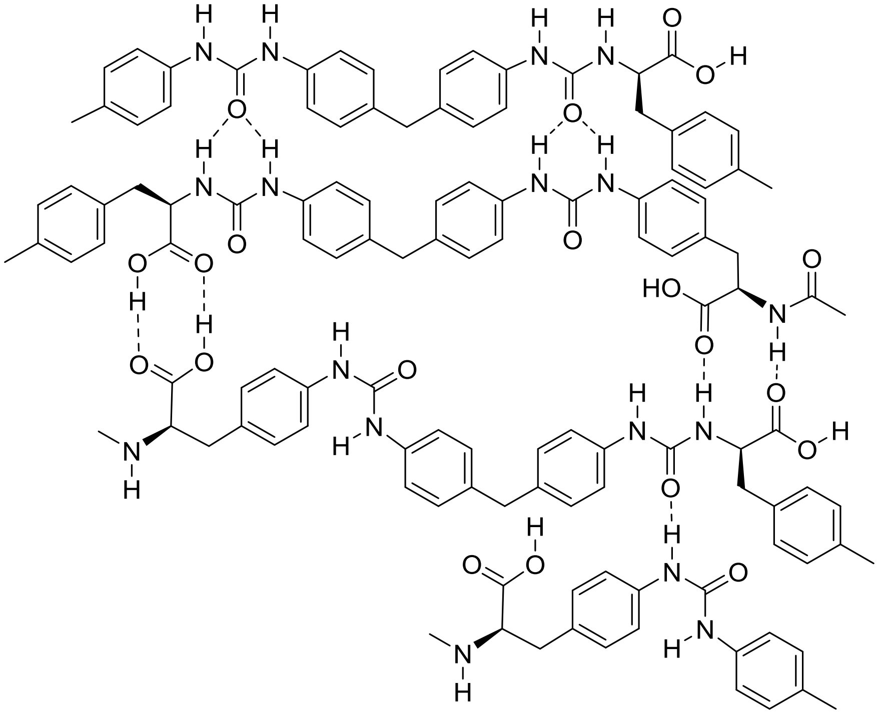


Figure 49. Representation of possible intermolecular hydrogen bonding between polyureas.

The mechanical properties such as tensile strength, Young's modulus, elongation at break, and strain energy density were determined using film and fibers in a tensile mode at room temperature (Table 4). We first compared the structural effects of carboxylate esterification on the mechanical properties of PUs using films whose values are shown in parentheses in Table 4. The films of PU-*X*a had higher mechanical strength at break and Young's moduli than those of PU-*X*b, presumably due to the stronger interchain interaction through hydrogen bonding of carboxylic acids, as mentioned above. Mechanical strength at maximum of PU-*X*a films ranged from 43 to 54 MPa while Young's moduli ranged 1.2-1.3 GPa, and elongation at break of 5.4-7.4 %. In order to improve the mechanical performance, we processed the fiber of PU-*X*a, and found that the values of fibers were higher than those of films. The fibers of PU-1a, PU-2a and PU-4a were stronger than PU-3a, presumably due to the rigid structure from para-substituted phenylenes. PU-4a was strongest owing to most rigid structures. Some of PUs were ductile and then strain energy density, *U*, is over 10 MJ/m^3. The fibers of PU-1a, PU-2a and PU-4a were stronger than conventional bioplastics and popular plastics such as PC and PMMA. The U value is also higher than those of ductile bio-derived polyimides (U: 4.95 J/cm^3) reported before (42), demonstrating PUs' excellent toughness. Also, in some way, this research also promotes the thoughtful usage of bioresources for the development of high-performance polymers in order to achieve the ultimate goal of sustainable future (49, 50).

2.4 Conclusion

A series of bio-based polyureas based on 4-aminophenylalanine (available from genetically modified *E. coli*) and its methyl ester were synthesized. All the PUs showed higher thermal resistance; T_g values above 100 °C. Some of PUs were processed into films and fibers and showed high mechanical strength of 156 MPa at maximum, which was much higher than conventional bio-based plastics. Especially fibers of PUs having carboxylic side chains polymerized with MDI and 1,4-PDI were very ductile showing high strain energy density above 10 MJ/m^3. Thus we clarified the high worth of unused amino acid, 4-APhe, as a monomer of PUs. Some of PU characteristics will make them a suitable candidate in the plastic industry for various applications.

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Chapter 3

Syntheses and characterization of aromatic polyimides from 4-aminophenylalanine as diamino acid monomer

Chapter 3

3.1 Introduction

The development of biobased polymers is indispensable for the establishment of a sustainable low-carbon society. A number of aliphatic biobased polymers such as polyesters; poly(lactic acid), (1) poly(hydroxyalkanote)s, (2) and poly(butylenes succinate), (3) and polyamides (polyamide 11 and polyamide 66) have been developed, but their low glass transition temperature, T_g, and only a small percentage of their substitutes limited their use for various applications as super-engineering plastic. The introduction of an aromatic component into the thermoplastic polymer backbone is an efficient method to intrinsically improve the material performance. (4,5) Although Kaneko research group have developed aromatic biobased polyesters from hydroxycinnamate derivatives such as p-coumaric acid (4-hydroxycinnamic acid, 4HCA), (6,7) caffeic acid, (3,4-dihydroxycinnamic acid, DHCA), (8) and ferulic acid (3-methoxy-4-hydroxycinnamic acid, MHCA), (9) their T_g ranged to 169 °C at most. (10) In order to apply them to the advanced fields of electronics, transportation vehicles, or aviation materials, a very high value of T_g over 200 °C in neat polymer materials without any additives is required. From this point of view, a high mechanical strength and Young's modulus are also necessary. One of the most advanced classes of high-performance polymeric materials is the polyimides (PIs), with excellent thermo-mechanical performance, high chemical stability, low coefficient of thermal expansion, etc. (11,12) The ability of polyimides to maintain their excellent physical, mechanical and electrical properties over a wide range of temperature has opened new design and application areas in the world of synthetic polymers (13-23). In fact, polyimides possess unique combination and excellent balance of properties that make them ideal candidates for a diverse range of applications such as high-temperature plastics, adhesives, photoresists, dielectrics, displays, membrane materials for separation, electroluminescence devices, fiber-optics, aircrafts and space-crafts materials (24-42).

Here, we used the microorganism-derived aromatic diamine, 4-aminophenylalanine (4-APhe) to prepare bioderived PIs based on its reaction with various tetra-carboxylic dianhydrides, and introduced different moieties in the intermediates to prepare bio-derived high performance PIs with high thermomechanical properties, high transparency, and good cell-compatibility. Polyimides, with tailored functional groups, prepared this way have advantage over a large number of available polyimides so far. The present work describes the approach covering every facet of polyimide sciences: syntheses, reactions, structures, physical properties especially thermal and mechanical properties, and functions.

The most widely practiced procedure in polyimide synthesis (43-50) is the two-step poly(amic acid) process. It involves reacting a dianhydride and a diamine at ambient conditions in a dipolar aprotic solvent such as N,N-dimethylacetamide (DMAc) or N-methylpyrrolidinone (NMP) to yield the corresponding poly(amic acid), which is then cyclized into the final polyimide.

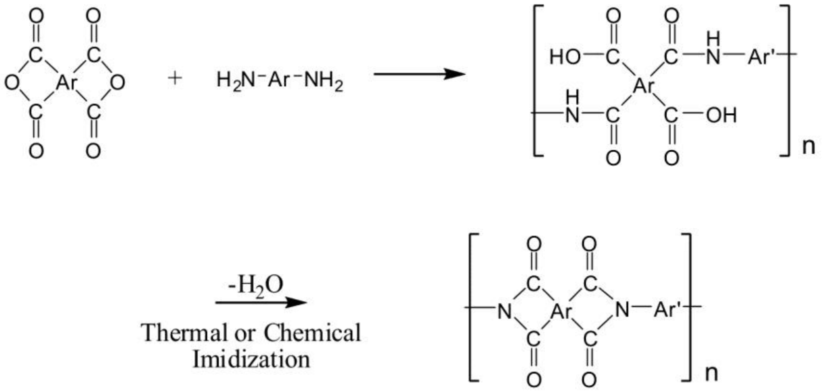


Figure 1: Two-step process synthesis for polyimides.

3.2 Experimental

3.2.1 Materials used in the present research

Dianhydrides such as pyromellitic dianhydride (PMDA: from TCI), 1,2,3,4-tetracarboxycyclobutane dianhydride (CBDA: from Aldrich), 3,3′,4,4′-benzophenone tetracarboxylic dianhydride (BTDA: from Aldrich), 4,4′-oxidiphthalic anhydride (OPDA: from Aldrich), 3,4,3′,4′-biphenyltetracarboxylic dianhydride (BPDA: from Aldrich), and

3,3′,4,4′-diphenylsulfone tetracarboxylic dianhydride (DSDA: from Aldrich) were recrystallized in acetic anhydride by refluxing for 5 h and then cooling to 0−5 °C. The crystals were carefully collected by filtration, washed in hot dioxane, and dried in vacuo. Trimethylsilyl chloride (TMSCl: from Aldrich), and *N,N*-dimethylacetamide (DMAc: 99.8% anhydrous from Kanto chemical) were used as received. 4-APhe hydrochloride salt used as a bio-based material for the PU syntheses was purchased from Watanabe Chemical Ind. Ltd. (Tokyo, Japan).

3.2.2 Measurements

Nuclear magnetic resonance (NMR) measurements such as ^{1}H-NMR and ^{13}C-NMR were performed by Bruker biospin AG 400 MHz, 54 mm spectrometer using DMSO-d_6 as a solvent. Specimen with a concentration of 0.5 mM in DMSO-d_6 in NMR test tubes of borosilicate glass (Wako Pure Chemical Industries, Φ 4.932-4.970 × 7 in.) was used.

The Fourier-transformed infrared (FT-IR) spectra were recorded with a Perkin-Elmer Spectrum One spectrometer in a wavenumber range between 4000 and 600 cm^{-1} using a diamond-attenuated total reflection (ATR) accessory.

The mass spectra were measured using a Fourier-transformed ion cyclotron resonance mass spectrometer (FT-ICR MS, Solarix) with an external ESI source (Analytica of Branford Inc.). The sample solution was infused into the ESI source in a positive ion mode with a syringe pump at 60 mL/h and was desolvated by a countercurrent nitrogen gas heated to 250 ^{o}C, and scanned from *m/z* 150 to *m/z* 1000.

The tensile measurements were carried out at an elongation speed of 0.5 mm/min on a tensiometer (Instron 3365, Kawasaki, Japan) at room temperature using polymer rectangle film with a length of 3 cm, a width of 1 cm and a thickness of 5 µm.

The number average molecular weight (*M*n), weight average molecular weight (*M*w) and molecular weight distribution (PDI) of the polymers were determined by gel permeation chromatography (GPC; Shodex GPC-101 with a tandem connection column system of KD-

803 and KD-807 (Shodex, Tokyo, Japan)) after calibration with pullulan standards in dimethylformamide (DMF) as eluent at a flow rate of 0.5 ml min^{-1} using the RI signal detector (shodex, Tokyo, Japan). The concentration of sample used was 5 g/L, filtered after stirring overnight.

The ultraviolet-visible (UV-vis) transmission spectra were recorded by Perkin Elmer, Lambda 25 UV/Vis spectrometer at room temperature over the range of 200-800 nm in a quartz cell (1 cm × 1 cm) under Deuterium and Halogen lamp as light source.

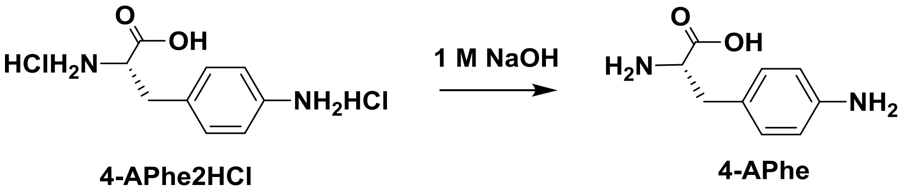
The solubility of the polymers was investigated using 1 mg of sample in 1mL of solvent at room temperature or at 50 °C.

Differential scanning calorimetry (DSC) of dried polymer samples (5-8 mg) was carried out under a nitrogen atmosphere at a scanning rate of 10 ^{o}C/min between 0 and 350 ^{o}C (Seiko Instruments SII, X-DSC7000T).

Thermogravimetry (TGA; SSC/5200 SII Seiko Instruments Inc.) was carried out to determine weight-loss temperature by heating from 30 to 800 °C at a rate of 5 °C/min under a nitrogen atmosphere. The polymer specimens for these thermal measurements were dried at 100 °C for 1 h to remove absorbed moisture and remained solvents before measurements.

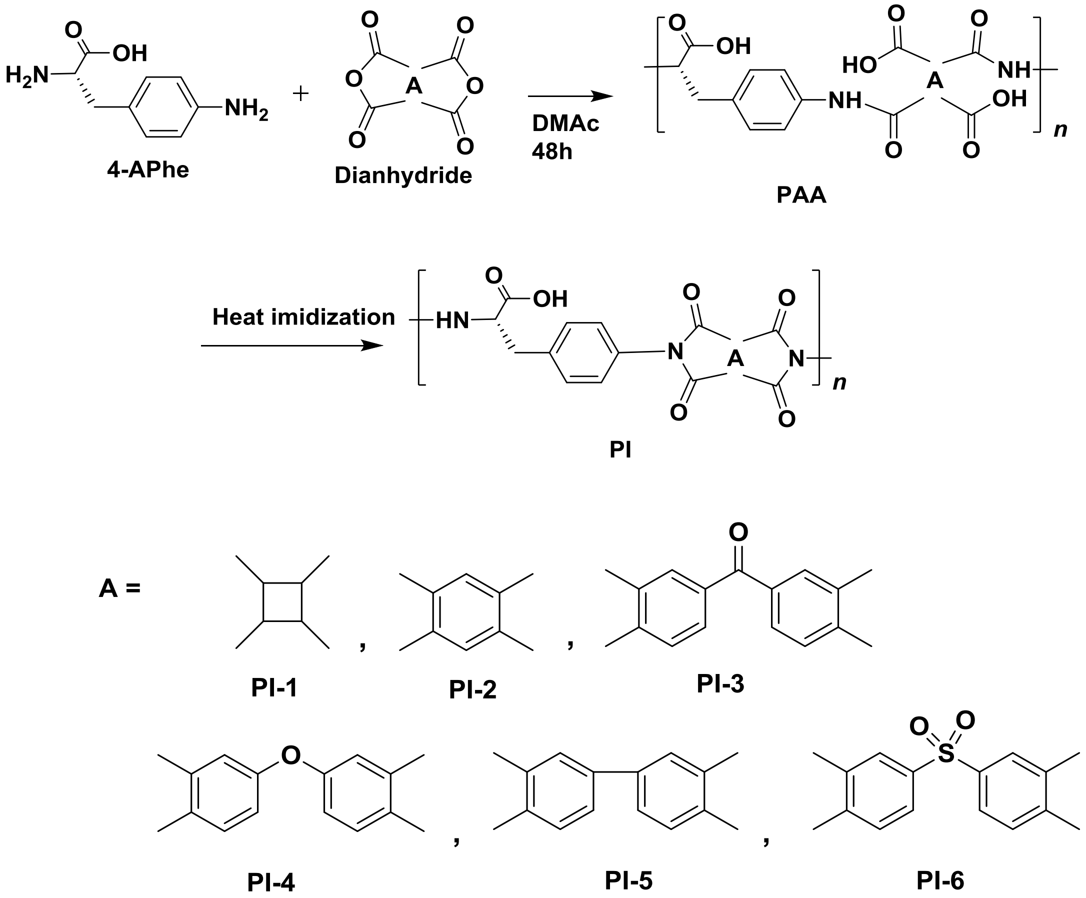
3.2.3 Syntheses

3.2.3.1 Monomer preparation



Scheme 3.1. Syntheses of 4-aminophenylalanine.

A series of polyimides (PIs) were synthesized by a reaction of diamine, 4-APhe with equimolar amounts of diisocyanates as shown in scheme 2.



Scheme 3.2. Syntheses of bio-based aromatic polyimides derived from 4-aminophenylalanine as diamine monomer. A means dianhydride moiety drawn at bottom where structures are numbered from 1 to 6.

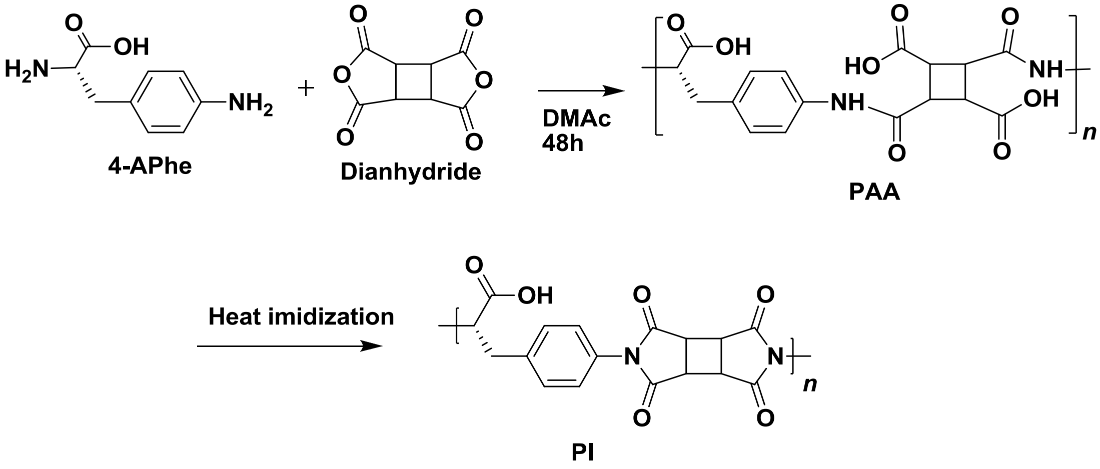
Synthesis of 4-aminophenylalanine (4-APhe). 4-APhe was synthesized (Scheme 1) by neutralization of 4-aminophenylalanine dihydrochloride by the procedure below. 4-APhe.2HCl (5.0 g, 19.8 mmol) was dissolved in 100 mL of ethanol to prepare white suspension. 1 M NaOH aq (40 mL, 40.0 mmol) was added dropwise into the suspension at 0 °C to turn into colorless homogeneous solution. By the addition of more NaOH aq, white precipitates of 4-APhe appeared over pH 9. The white precipitates of 4-APhe formed are separated by filtration and dried in vacuo overnight at temperature 60 °C (yield: 2.1 g, 59 %). The product was highly hygroscopic. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.60-

2.66 (dd, 1H, *β*-methylene), 2.94-2.98 (dd, 1H, *α*-methylene), 3.20-3.23 (dd, 1H, methine), 4.89 (s, 2H, amino), 6.46-6.48 (d, 2H, ortho-hydrogens of phenylene), 6.88-6.90 (d, 2H, meta-hydrogens of phenylene). ^{13}C-NMR (100 MHz, DMSO-d_6, δ, ppm): 35.5, 56.0, 116.8, 125.5, 130.2, 145.4, 174.1. FT-ICR MS (ESI) calcd for [M-H$^+$, $C_9H_{11}N_2O_2$]$^-$: calculated value 179.0826, observed value 179.0825.

3.2.3.2 Polyimide syntheses

A detailed procedure with relevant characterizations for each polymer is explained as below.

10. **Synthesis of polyimide (4APhe-CBDA) using 1,2,3,4-tetracarboxycyclobutane dianhydride:**



Scheme 3.3. Syntheses of polyimide (4-APhe-CBDA).

Procedure:

DMAc (0.5 ml) was added to 4APhe (100 mg, 0.55 mmol) under nitrogen atmosphere and the mixture was stirred at room temperature. Then 1,2,3,4-tetracarboxycyclobutane dianhydride (CBDA, 121 mg, 0.55 mmol) was added, and the mixture was stirred for 40 hours. The reaction solution was added dropwise to distilled water to re-precipitate the polymer. The precipitate was filtered, washed with methanol and vacuum dried to obtain yellow fibrillar polyamic acid (4APhe-CBDA).

Yield: 992 mg, yield: 90%

Number average molecular weight M_n: 2.53 x 10^5

Weight average molecular weight M_w: 3.71 x 10^5

PDI: 1.38

^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.68-2.96 (d, 2H, methylene), 2.79(s, 1H, methine), 3.40-3.57(4H, cyclobutane ring), 7.26-7.57 (4H, Aromatic ring), 8.85(s, 1H, amide), 9.86 (s, 1H, amide), 13.50 (2H, carboxylic).

^{13}C NMR (100 MHz, DMSO-d_6): δ 34.1, 34.9, 37.9, 40.6, 53.8, 123.1, 125.2, 127.4, 129.7, 130.4, 132.4, 165.8, 166.1, 172.0, 172.4.

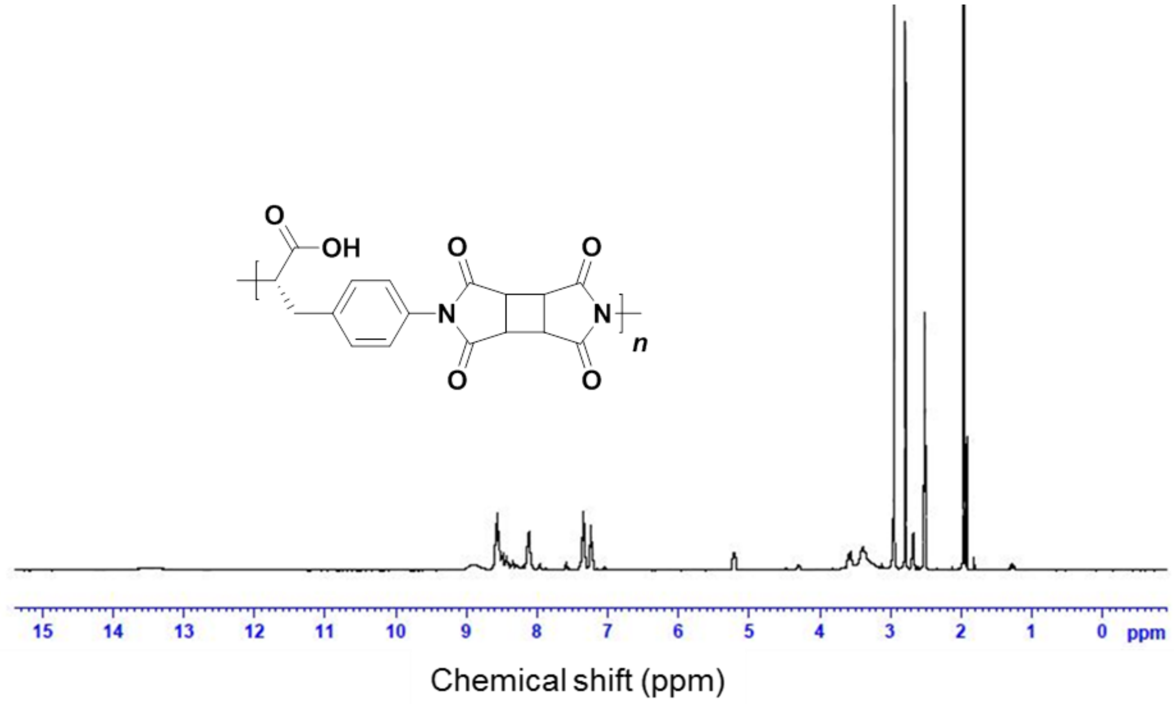


Figure 2: ^{1}H-NMR of poly(amic acid) (4APhe-CBDA).

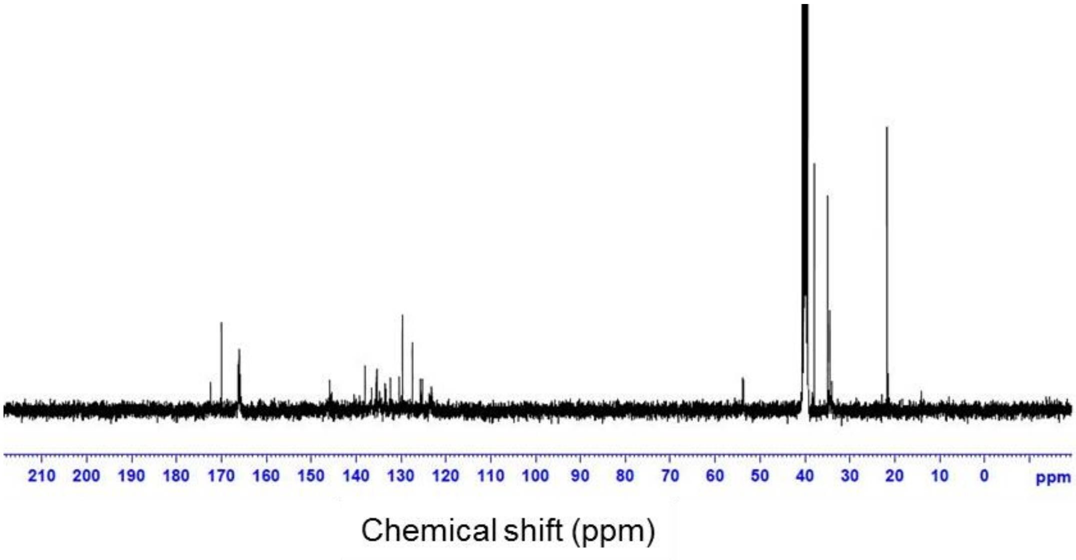


Figure 3: ^{13}C-NMR of poly(amic acid) (4APhe-CBDA).

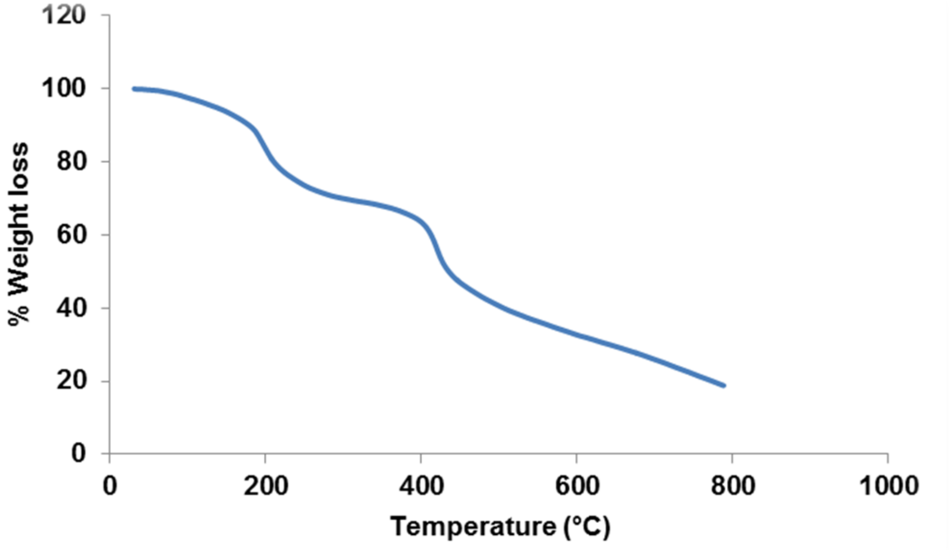
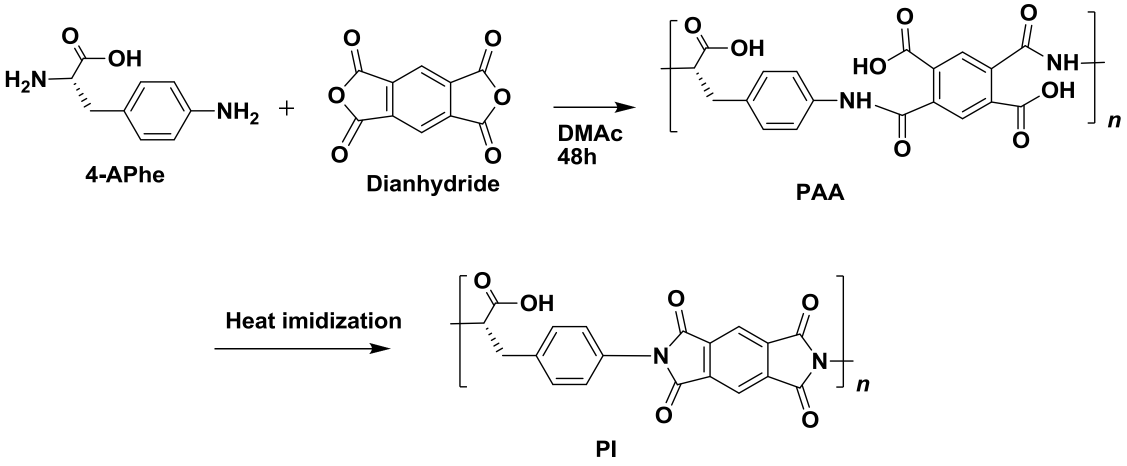


Figure 4: TGA curve of poly(amic acid) (4APhe-CBDA).

11. **Synthesis of polyimide (4APhe-PMDA) using pyromellitic dianhydride (PMDA):**



Scheme 3.4. Syntheses of polyimide (4-APhe-PMDA).

Procedure:

DMAc (0.5 ml) was added to 4APhe (100 mg, 0.55 mmol) under nitrogen atmosphere and the mixture was stirred at room temperature. Then pyromellitic dianhydride (PMDA, 121 mg, 0.55 mmol) was added, and the mixture was stirred for 40 hours. The reaction solution was added dropwise to distilled water to re-precipitate the polymer. The precipitate was filtered, washed with methanol and vacuum dried to obtain yellow crystalline polyamic acid (4APhe-PMDA).

Yield: 184 mg, yield: 87.67 %

Number average molecular weight M_n: 1.18 x 10^5

Weight average molecular weight M_w: 1.76 x 10^5

PDI: 1.494

^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.96 (d, 2H, methylene), 2.79(s, 1H, methine), 7.12-7.61 (4H, aromatic ring), 8.01-8.48 (2H, aromatic ring), 8.57 (s, 1H, amide), 8.97 (s, 1H, amide), 11.08(2H, carboxylic).

^{13}C NMR (100 MHz, DMSO-d_6): δ 39.4, 40.6, 121.7, 128.3, 133.4, 134.4, 135.2 167.8, 173.0, 182.4.

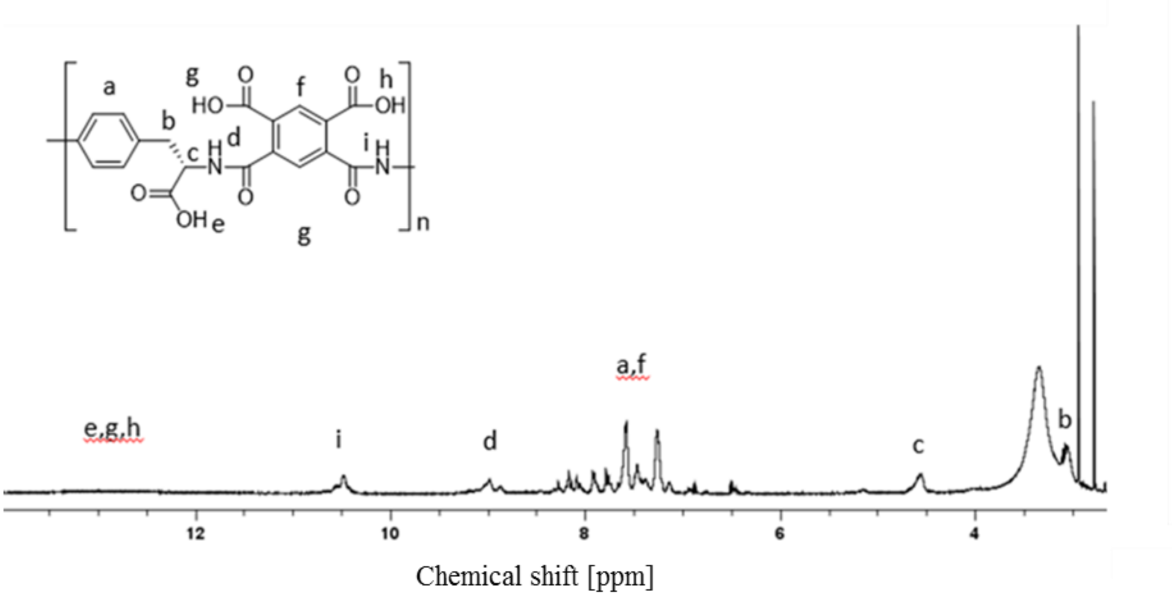


Figure 5: ^{1}H-NMR of poly(amic acid) (4APhe-PMDA).

Figure 6: Physical appearance of poly(amic acid) (4APhe-PMDA).

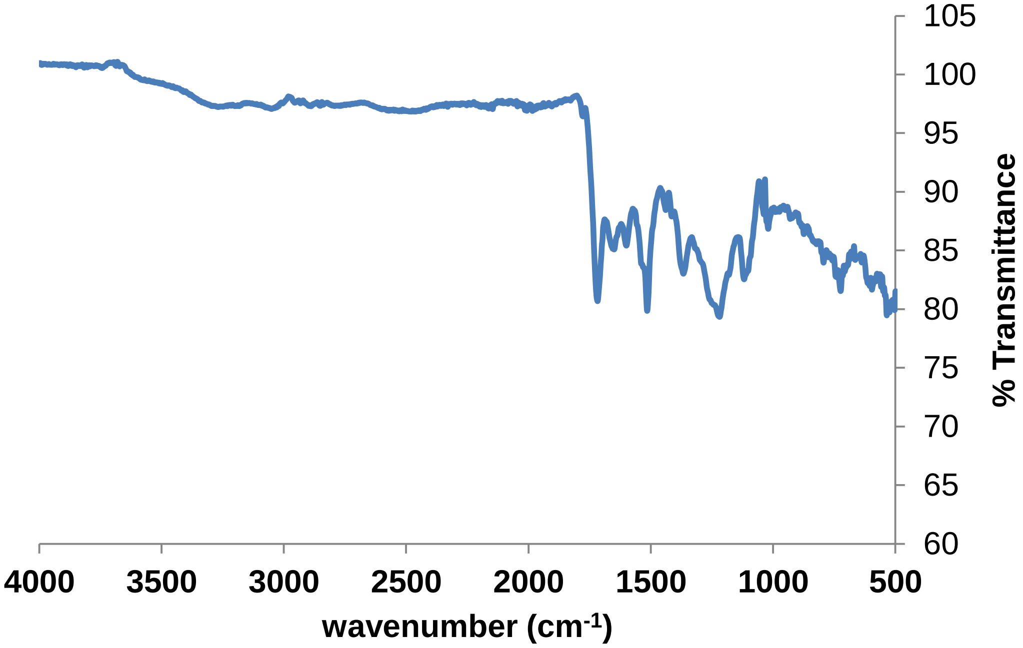


Figure 7: IR spectra of poly(amic acid) (4APhe-PMDA).

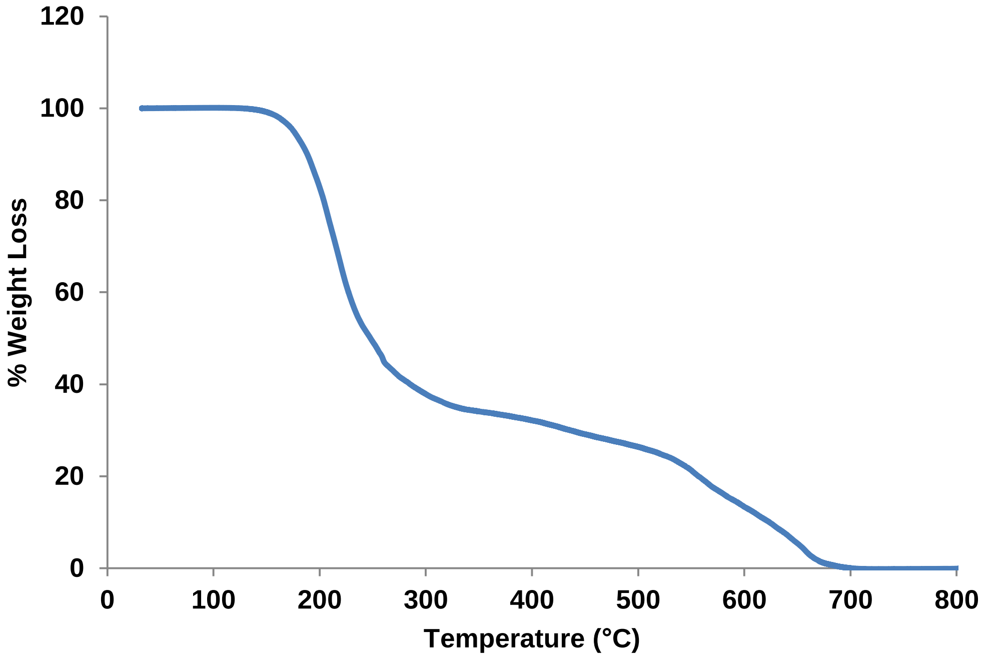


Figure 8: TGA pattern of poly(amic acid) (4APhe-PMDA).

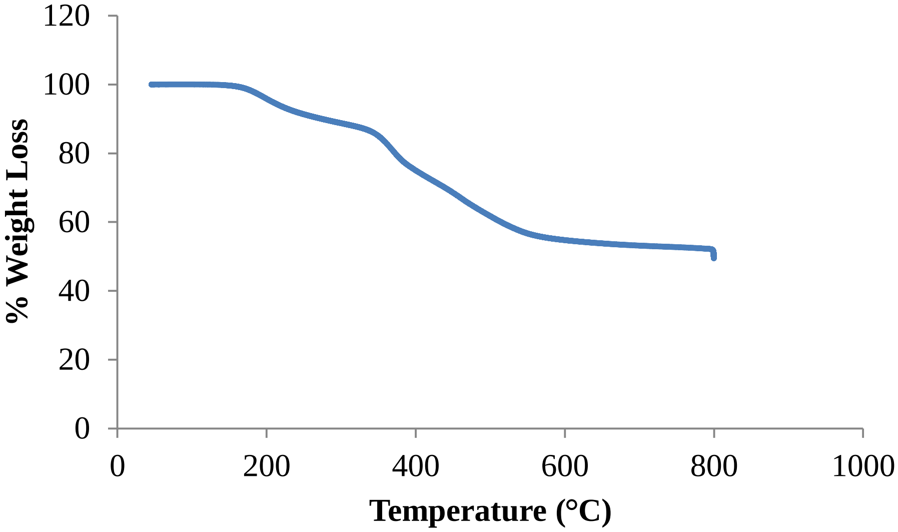
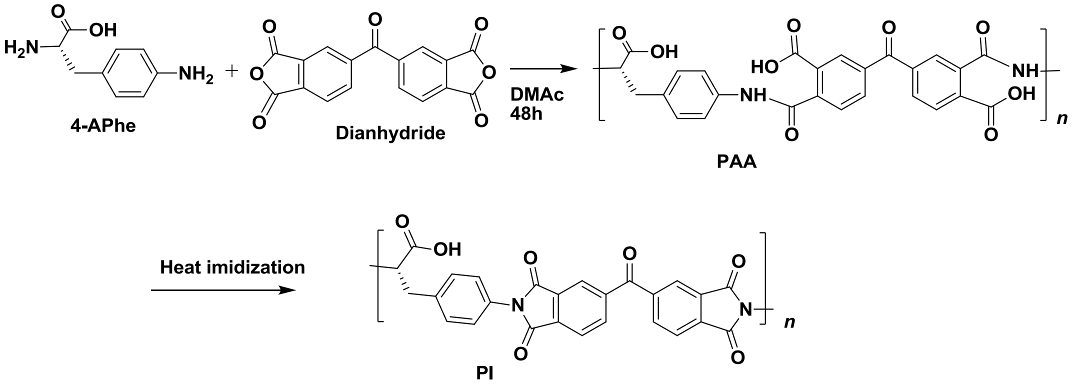


Figure 9: TGA pattern of polyimide (4APhe-PMDA).

12. **Synthesis of polyimide (4APhe-BTDA) using 3,3’,4,4’-benzophenone tetracarboxylic dianhydride (BTDA):**



Scheme 3.5. Syntheses of polyimide (4-APhe-BTDA).

Procedure

DMAc (0.5 ml) was added to 4APhe (100 mg, 0.55 mmol) under nitrogen atmosphere and the mixture was stirred at room temperature. Then pyromellitic dianhydride (BTDA, 121 mg, 0.55 mmol) was added, and the mixture was stirred for 40 hours. The reaction solution was added dropwise to distilled water to re-precipitate the polymer. The precipitate was filtered, washed with methanol and vacuum dried to obtain yellow polyamic acid (4APhe-BTDA).

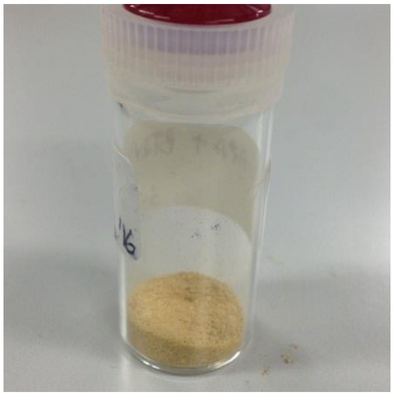


Figure 10: Physical appearance of poly(amic acid) (4-APhe-BTDA).

Yield: 250 mg, yield: 96 %

Number average molecular weight M_n: 2.50 x 10^5

Weight average molecular weight M_w: 4.04 x 10^5

PDI: 1.62

^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.96 (d, 2H, methylene), 2.80 (s, 1H, methine), 7.11-8.19 (9H, aromatic ring), 8.99 (s, 1H, amide), 10.48 (s, 1H, amide), 12.85 (1H, carboxylic).

^{13}C NMR (100 MHz, DMSO-d_6): δ 39.4, 40.6, 121.7, 128.3, 133.4, 134.4, 135.2 167.8, 173.0, 182.4.

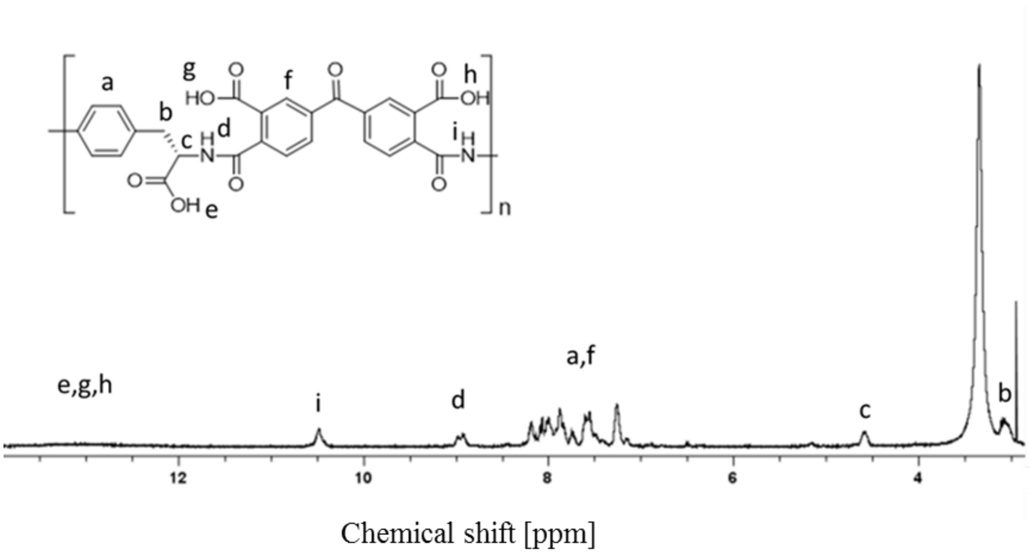
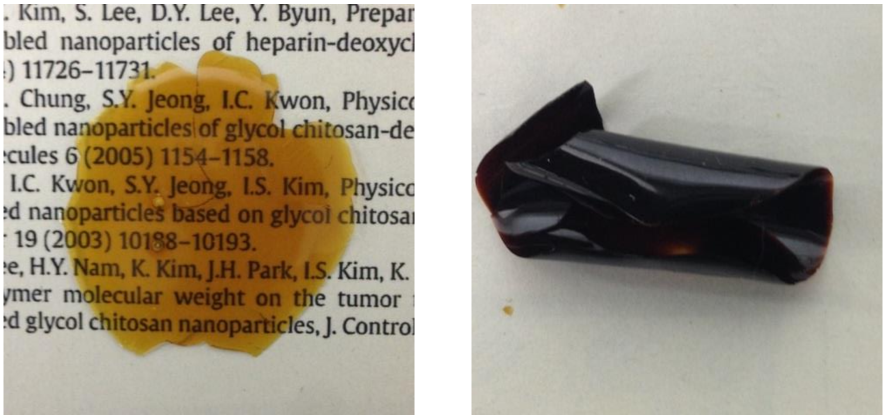


Figure 11: ^{1}H-NMR of poly(amic acid) (4-APhe-BTDA).



a. b.

Figure 12. a) Poly(amic acid) film, b) Polyimide film.

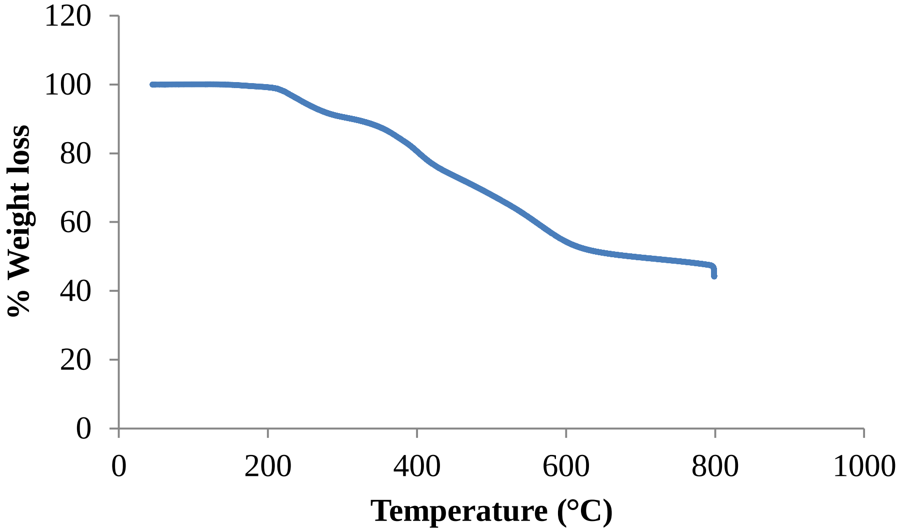


Figure 13: TGA pattern by poly(amic acid) (4-APhe-BTDA).

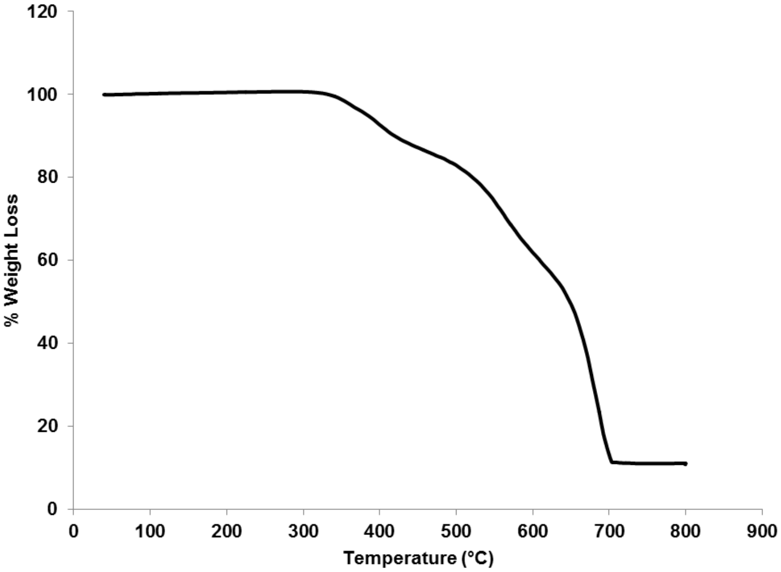


Figure 14: TGA pattern by polyimide (4-APhe-BTDA).

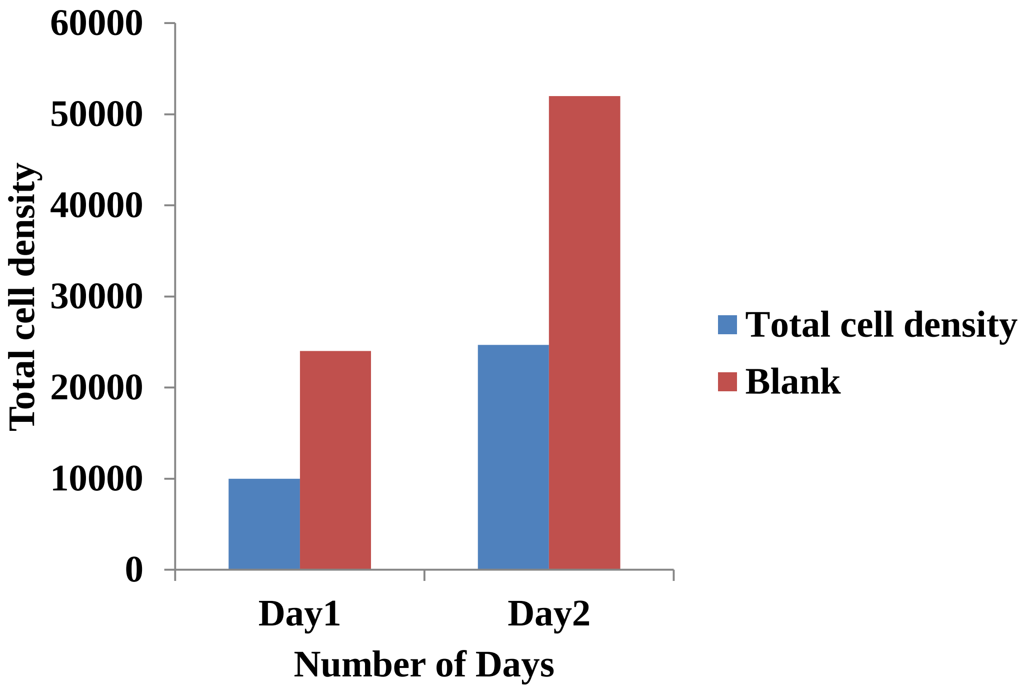
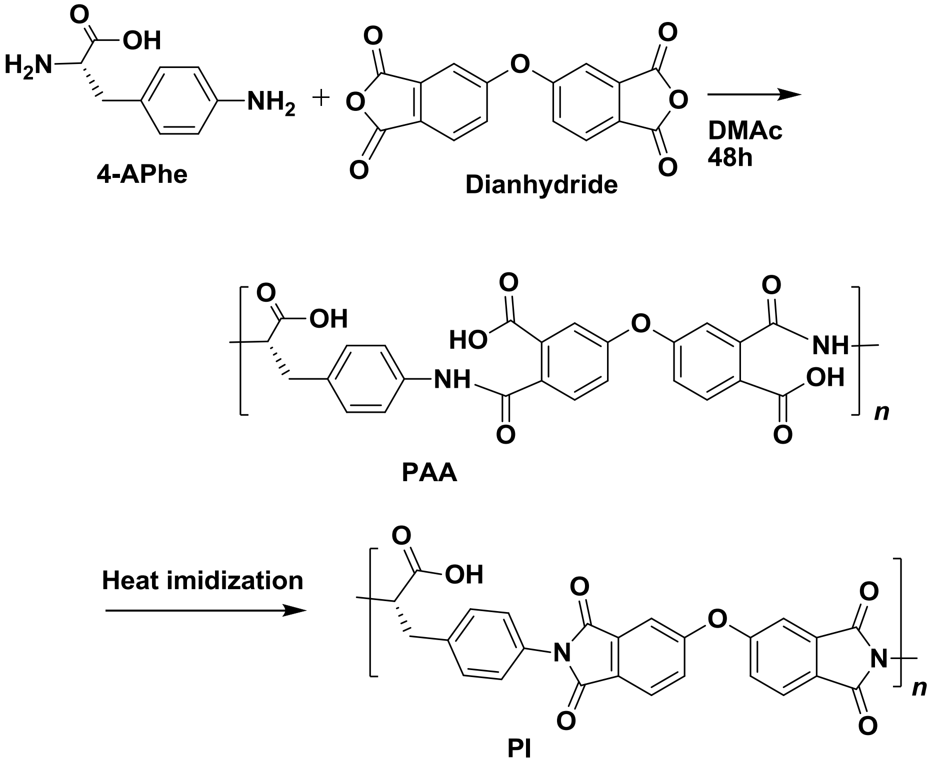


Figure 15: cell culturing experiment.

Day1	Cell density	Total cells
Blank	12000	24000
Well1	5000	10000
Well2	5000	10000
Well3	5000	10000
Day 2	**Cell density**	**Total cells**
Blank	26000	37500
Well1	13000	26000
Well2	12000	24000
Well3	12000	24000

Table 2.1: Cells count.

13. **Synthesis of polyimide (4APhe-OPDA) using 4,4’-oxidiphthalic anhydride (OPDA):**



Scheme 3.6. Syntheses of polyimide (4-APhe-OPDA).

Procedure:

DMAc (0.5 ml) was added to 4APhe (100 mg, 0.55 mmol) under nitrogen atmosphere and the mixture was stirred at room temperature. Then 4,4’-oxidiphthalic anhydride (OPDA, 172 mg, 0.55 mmol) was added, and the mixture was stirred for 40 hours. The reaction solution was added dropwise to distilled water to re-precipitate the polymer. The precipitate was filtered, washed with methanol and vacuum dried to obtain yellow polyamic acid (4APhe-OPDA).

Yield: 220 mg, yield: 95 %

Number average molecular weight M_n: 2.15 x 10^5

Weight average molecular weight M_w: 3.27 x 10^5

PDI: 1.52

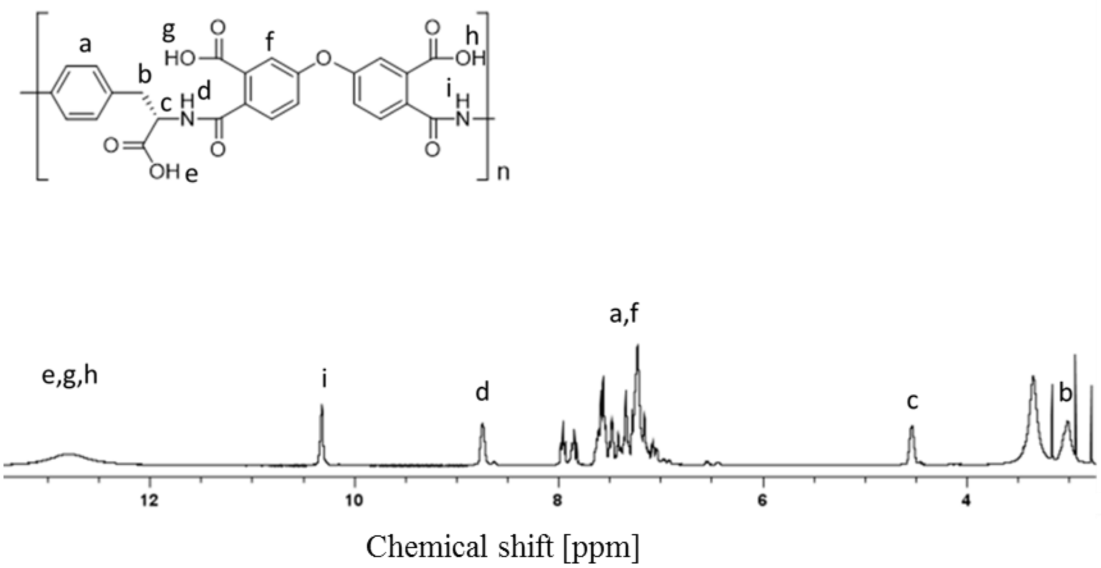


Figure 16: ^{1}H-NMR of poly(amic acid) (4-APhe-OPDA).

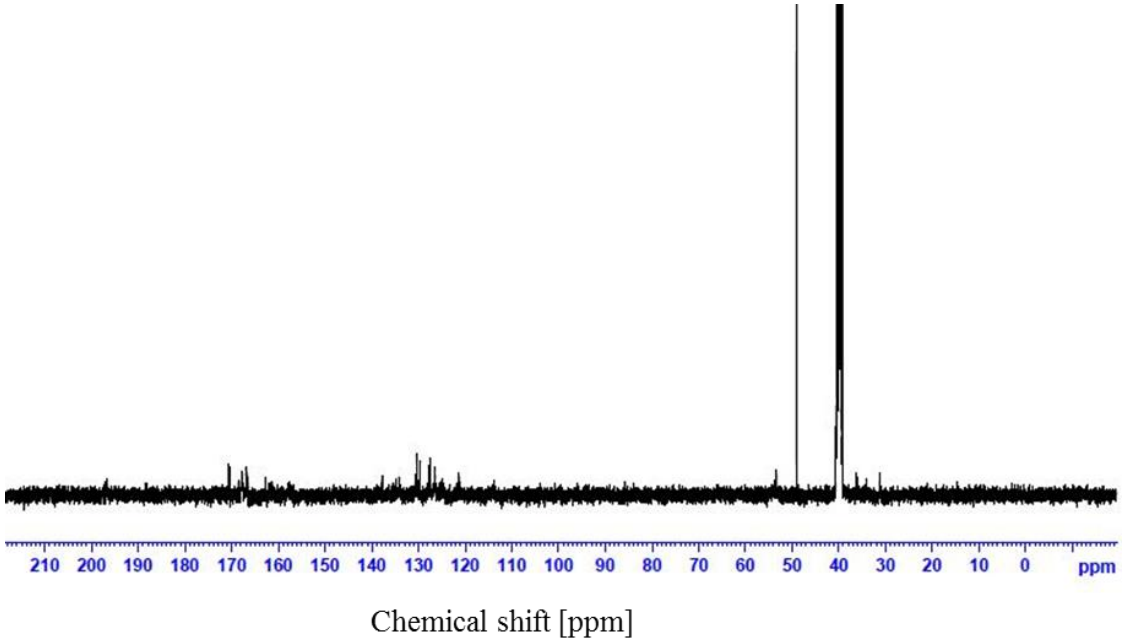


Figure 17: ^{13}C-NMR of poly(amic acid) (4-APhe-OPDA).

^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 2.74-2.96 (d, 2H, methylene), 2.85 (s, 1H, methine), 7.29-7.61 (10H, aromatic ring), 8.15 (s, 1H, amide), 8.82 (s, 1H, amide), 12.85 (1H, carboxylic).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 39.4, 40.3, 121.3, 121.4, 126.4, 127.5, 127.8, 129.6, 130.4, 130.6, 137.6, 166.7, 167.0, 167.7, 170.4, 170.8.

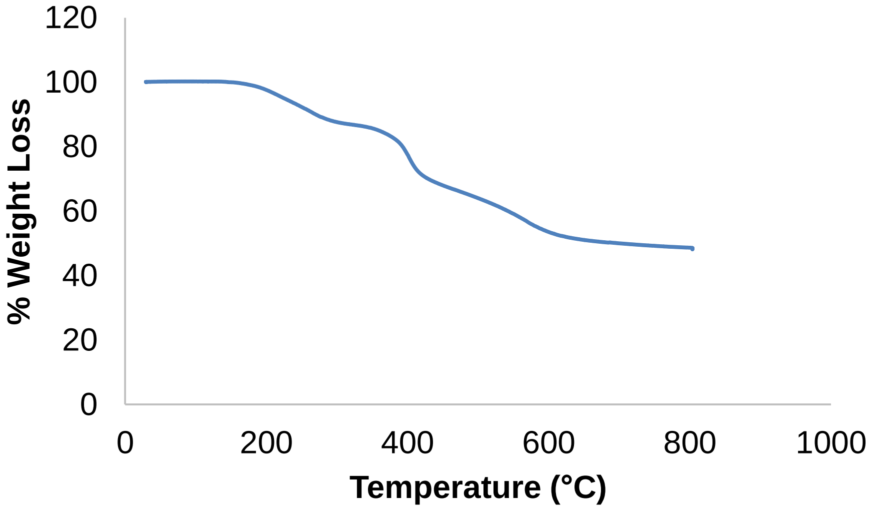


Figure 18: TGA curve of polyamic acid (4-APhe-OPDA).

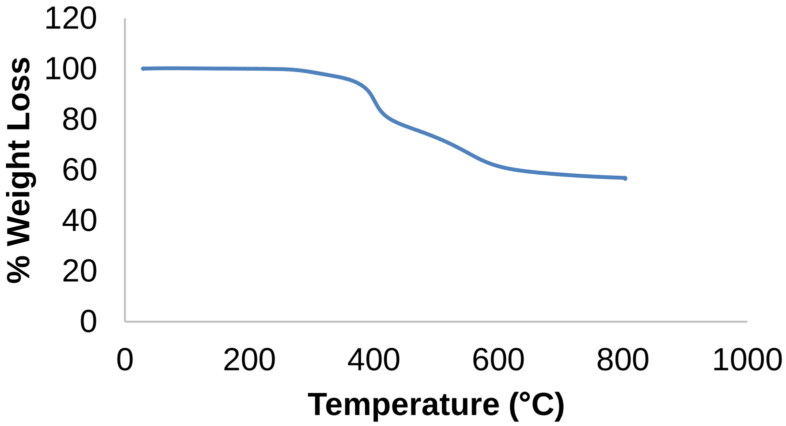
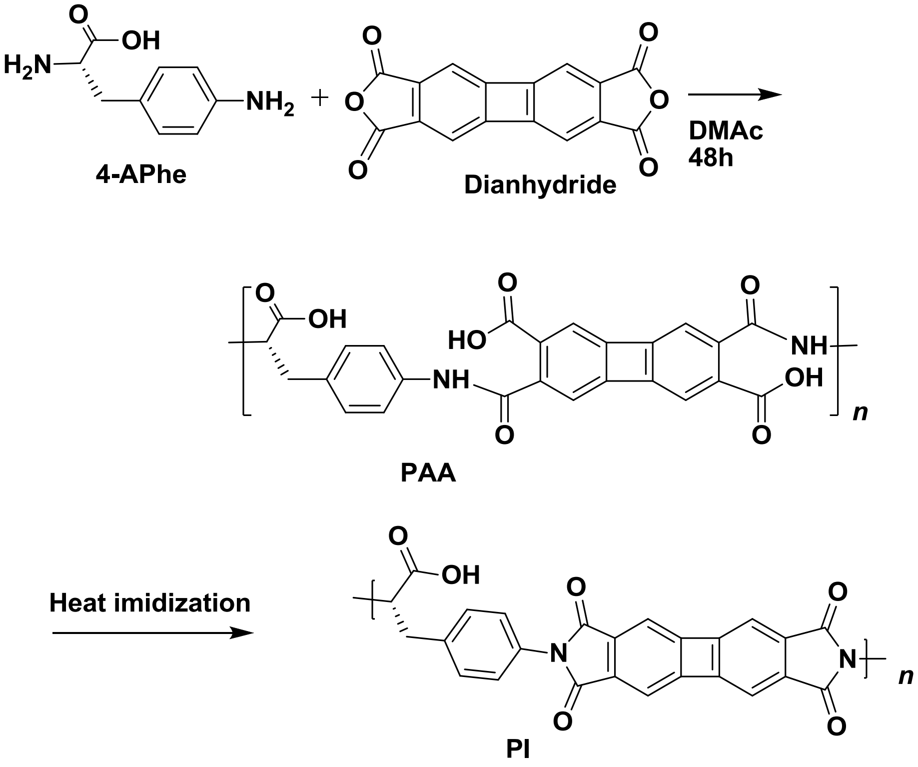


Figure 19: TGA curve of polyimide (4-APhe-OPDA).

14. **Synthesis of polyimide (4APhe-BPDA) using 3,4',3,4'-biphenyltetracarboxylic dianhydride (BPDA):**



Scheme 3.7. Syntheses of polyimide (4-APhe-BPDA).

Procedure:

DMAc (0.5 ml) was added to 4APhe (100 mg, 0.55 mmol) under nitrogen atmosphere and the mixture was stirred at room temperature. Then 4,4'-oxidiphthalic anhydride (BPDA, 163 mg, 0.55 mmol) was added, and the mixture was stirred for 40 hours. The reaction solution was added dropwise to distilled water to re-precipitate the polymer. The precipitate was filtered, washed with methanol and vacuum dried to obtain yellow polyamic acid (4APhe-BPDA).

Yield: 225 mg, yield: 93 %

Number average molecular weight M_n: 2.86 x 10^5

Weight average molecular weight M_w: 4.42 x 10^5

PDI: 1.54.

^{1}H-NMR (400 MHz, DMSO-d6): δ 2.96 (d, 2H, methylene), 2.80 (s, 1H, methine), 7.18-8.94 (8H, aromatic ring), 8.28 (s, 1H, amide), 10.45 (s, 1H, amide), 13.20 (1H, carboxylic).

^{13}C NMR (100 MHz, DMSO-d6): δ 39.3, 40.4, 119.9, 128.7, 129.3, 129.7, 130.7, 135.9, 140.8, 168.1, 168.1, 168.3, 170.0.

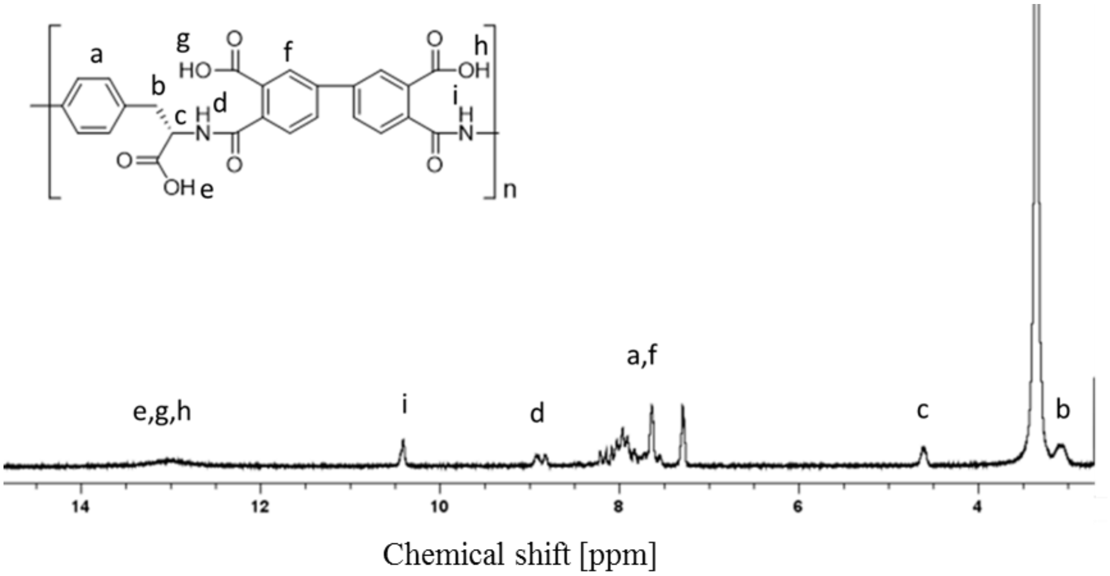


Figure 20: ^{1}H-NMR of poly(amic acid) (4-APhe-BPDA).

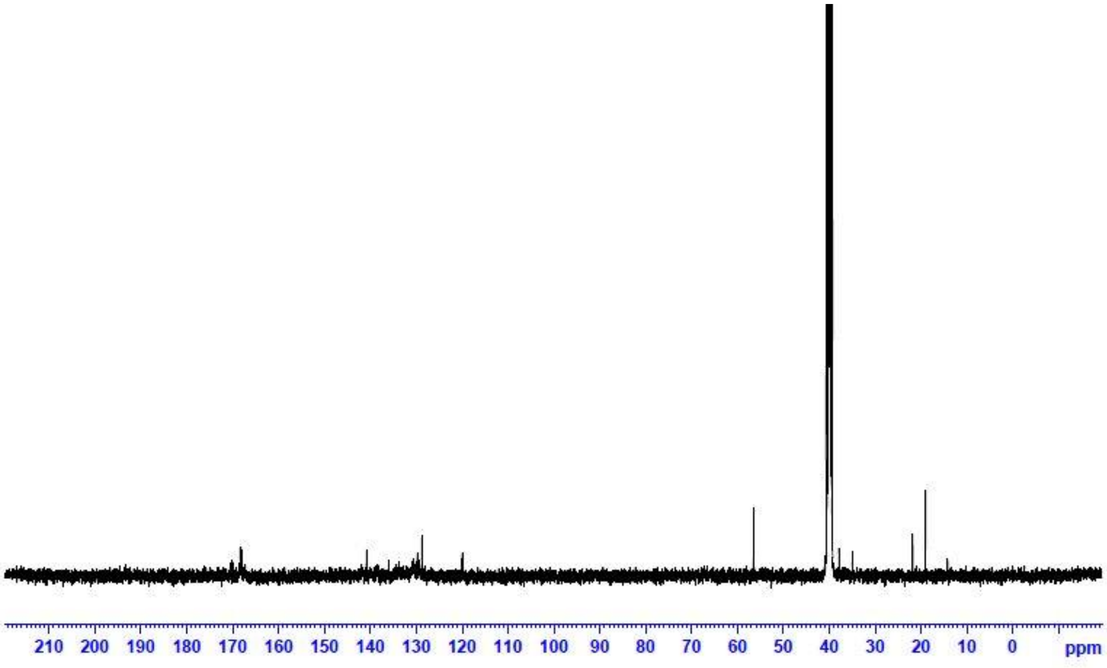


Figure 21: ^{13}C-NMR of poly(amic acid) (4-APhe-BPDA).

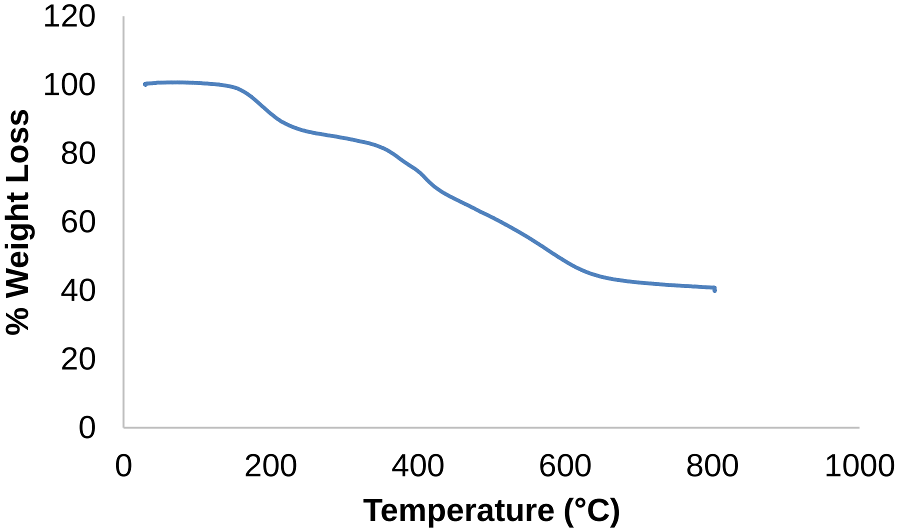
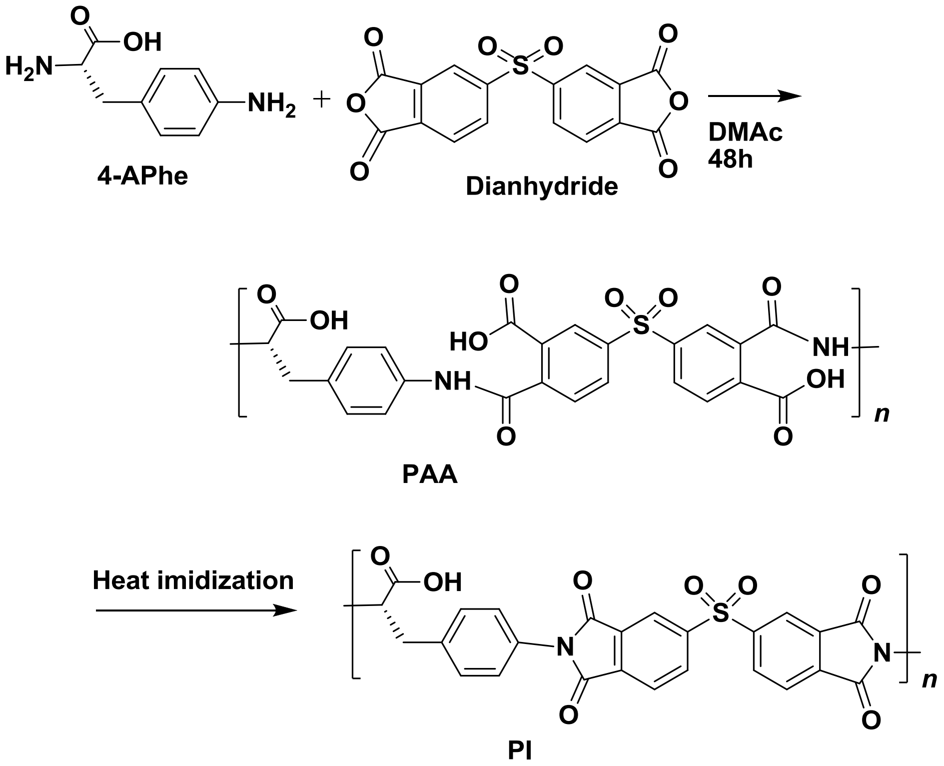


Figure 22: TGA curve of poly(amic acid) (4-APhe-BPDA).

15. **Synthesis of polyimide (4APhe-DSDA) using 3,3’,4,4’-diphenylsulphone tetracarboxylic dianhydride (DSDA):**



Scheme 3.8. Syntheses of polyimide (4-APhe-DSDA).

Procedure:

DMAc (0.5 ml) was added to 4APhe (100 mg, 0.55 mmol) under nitrogen atmosphere and the mixture was stirred at room temperature. Then 3,3’,4,4’-diphenylsulphone tetracarboxylic dianhydride (DSDA, 172 mg, 0.55 mmol) was added, and the mixture was stirred for 40 hours. The reaction solution was added dropwise to distilled water to re-precipitate the polymer. The precipitate was filtered, washed with methanol and vacuum dried to obtain yellow polyamic acid (4APhe-DSDA).

Yield: 250 mg, yield: 93 %

Number average molecular weight M_n: 2.43 x 10^5

Weight average molecular weight M_w: 3.73 x 10^5

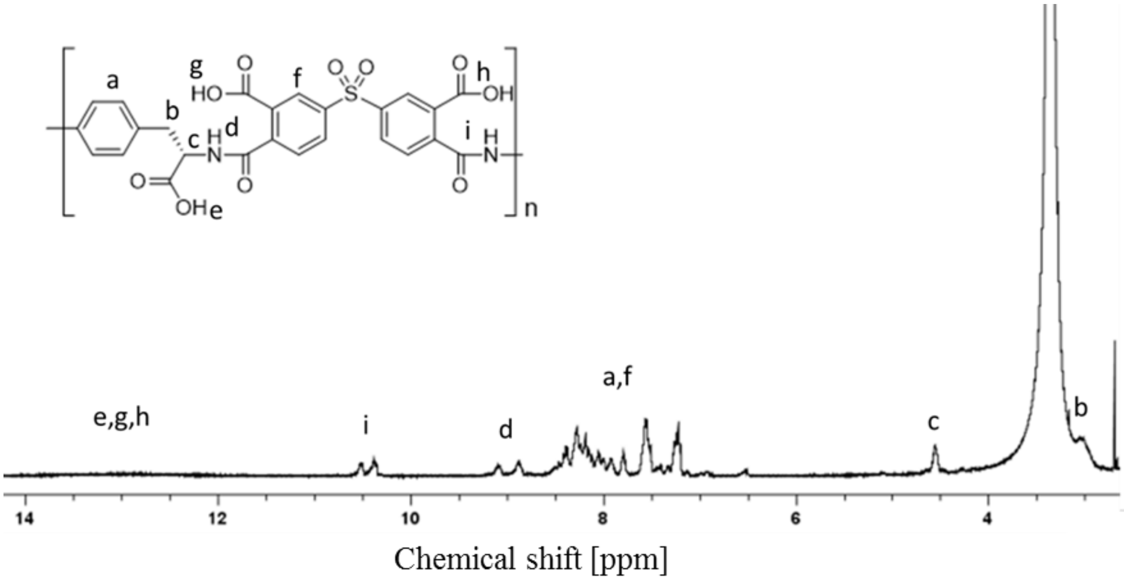


Figure 23: ^{1}H-NMR of poly(amic acid) (4-APhe-DSDA).

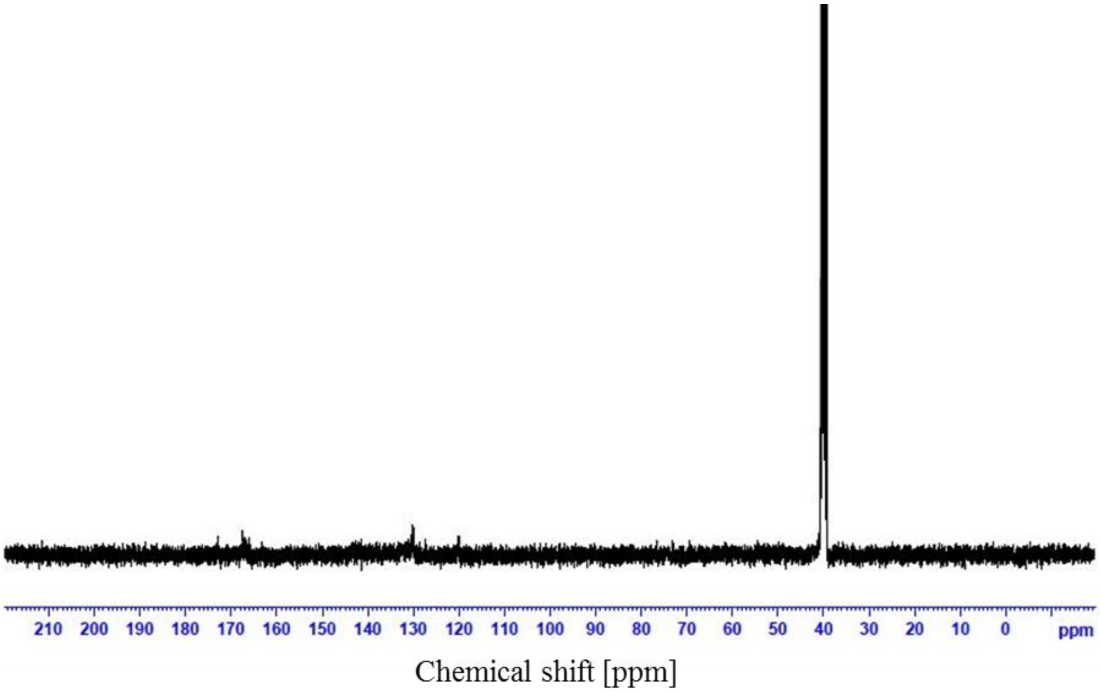


Figure 24: ^{13}C-NMR of poly(amic acid) (4-APhe-DSDA)..

PDI: 1.53.

^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 2.99 (d, 2H, methylene), 2.80 (s, 1H, methine), 7.25-8.51 (10H, aromatic ring), 8.24 (s, 1H, amide), 10.53 (s, 1H, amide), 13.20 (1H, carboxylic).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 39.3, 40.4, 119.9, 128.7, 129.3, 129.7, 130.7, 135.9, 140.8, 168.1, 168.1, 168.3, 170.0.

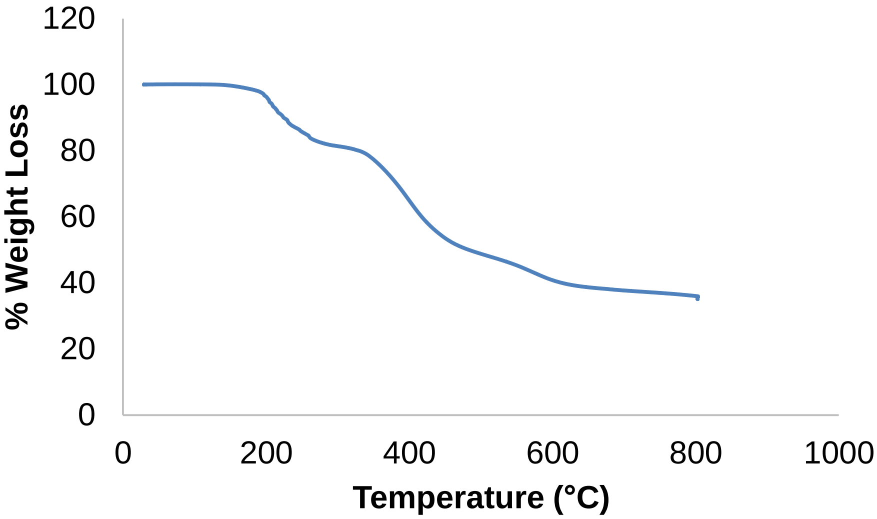


Figure 25: TGA curve of poly(amic acid) (4-APhe-DSDA).

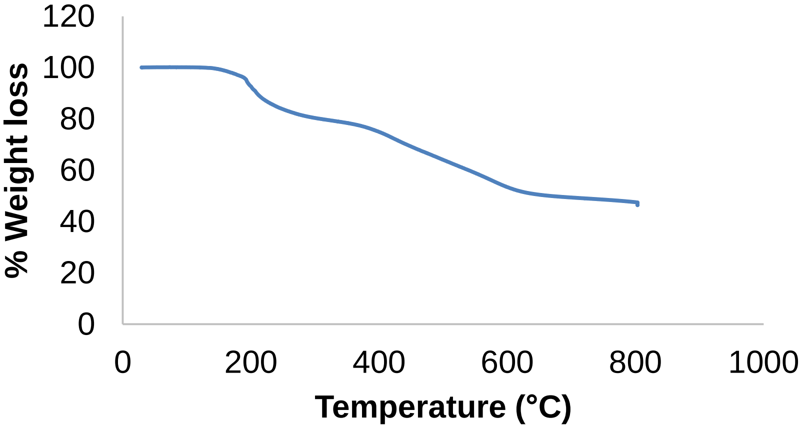


Figure 26: TGA curve of poly(amic acid) (4-APhe-DSDA).

3.3 Discussion

A series of PAAs were prepared by polycondensation of the micro-organism derived 4-aminophenylalanine (4-APhe) with various tetracarboxylic dianhydrides CBDA, PMDA, BTDA, OPDA, BPDA or DSDA (Scheme 2). The resulting PAAs were abbreviated as PAA-1 for CBDA based PAA, PAA-2 for PMDA based PAA, PAA-3 for BTDA based PAA, PAA-4 for OPDA based PAA, PAA-5 for BPDA based PAA, PAA-6 for DSDA based PAA as shown in Table1. The structures of the PAAs were confirmed by NMR study.

Table1. Summary of PAAs syntheses using 4APhe with dianhydrides.

polymers	diisocyanates	yield (%)	M_n ^{a} ($x10^5$)	M_w ^{a} ($x10^5$)	PDI ^{a}	film property
PAA-1	CBDA	87	1.72	2.80	1.63	Brittle
PAA-2	PMDA	92	1.32	2.08	1.57	Semi-ductile
PAA-3	BTDA	99	1.71	1.63	1.52	Ductile
PAA-4	OPDA	95	1.25	1.68	1.34	Ductile
PAA-5	DSDA	98	1.37	3.37	2.45	Ductile
PAA-6	BPDA	97	1.58	3.76	2.38	Semi-ductile

b) Molecular weight and its distribution was measured by gel permeation chromatography using external pullulan standards in dimethylformamide as eluent at a flow rate of 0.5 ml min^{-1} using the RI signal detector.

All the PAAs were soluble in DMAc solvent, and yellowish films with high-transparency were prepared by casting at 60-70 °C. The films were subsequently imidized to anneal stepwise at 100, 150, 200 and 250 °C for 1 h at each step in an oven, and imide ring formation was confirmed by IR spectroscopy. The resulting PIs were abbreviated as PI-1, PI-2, PI-3, PI-4, PI-5, PI-6. The PI films had a deeper yellow color or even brown color than the PAAs, which corresponded to a cut-off wavelength shift toward higher value by transmittance spectra.

M_w, M_n and PDI were determined using PAA polymers as PIs were not soluble in commonly used solvents. The results of GPC measurements are summarized in Table 1. PAAs had high M_w, and M_n values in the range of (1.18-2.86) × 10^5 and (1.76-4.42) × 10^5, respectively and PDI ranged from 1.38 to 1.62 (Table 1).

The solubilities of all the PAAs and PIs were investigated in various solvents as shown in Table 2.

Table 2: Solubilities of PAAs and PIs from 4-APhe and various dianhydrides in a series of organic solvents.

Sol. Polymer	H_2O	CH_3OH	*EtOH*	CH_3CN	*THF*	*Ether*	*DMF*	*DMAc*	*NMP*	*DMSO*	*EtOAc*	$CHCl_3$	*Hexane*	H_2SO_4
PAA-1	-	-	-	-	-	-	+	+	+	+	-	-	-	+
PI-1	-	-	-	-	-	-	-	-	-	-	-	-	-	+
PAA-2	-	-	-	-	-	-	+	+	+	+	-	-	-	+
PI-2	-	-	-	-	-	-	-	-	-	-	-	-	-	+
PAA-3	-	-	-	-	-	-	+	+	+	+	-	-	-	+
PI-3	-	-	-	-	-	-	-	-	-	-	-	-	-	+
PAA-4	-	-	-	-	-	-	+	+	+	+	-	-	-	+
PI-4	-	-	-	-	-	-	-	-	-	-	-	-	-	+
PAA-5	-	-	-	-	-	-	+	+	+	+	-	-	-	+
PI-5	-	-	-	-	-	-	-	-	-	-	-	-	-	+
PAA-6	-	-	-	-	-	-	+	+	+	+	-	-	-	+
PI-6	-	-	-	-	-	-	+	+	+	+	-	-	-	+

The solubility of the polymers was tested by dissolving them in three groups of solvents at room temperature. The three groups of solvents were: A) non-polar solvents such as hexane, benzene, toluene, dichloromethane (DCM), chloroform and diethyl ether, B) polar protic solvents such as distilled water, methanol, ethanol, and concentrated sulfuric acid, and C) polar aprotic solvents such as acetone, acetonitrile, ethyl acetate, THF, DMF, DMAc, NMP and DMSO. All of the PAAs prepared were soluble in polar aprotic solvents such as NMP, DMAc, DMF, and DMSO at room temperature, while PI were soluble only in trifluoroacetic acid and concentrated sulfuric acid.

The thermal degradation of all the synthesized PAAs and PIs were analyzed by thermogravimetric analysis (representative curves along with their polymers detailed description) in a nitrogen atmosphere at a heating rate of 10 °C min^{-1}, and the 5% and 10 % weight-loss temperatures (T_{d5} and T_{d10}) were determined. All the TGA values were summarized in Table 3. The PAAs samples were dried at 100 °C before measurement. TGA values were investigated upto 800 °C to evaluate the residual weight which was found to be nearly 5 % or less indication considerable degradation. The T_{d5} values lie mainly above 130 °C for most of the PAAs, and T_{d10} ranged from 176-290 °C. On the other hand, The T_{d5} values lie mainly above 224 °C for most of the PAAs, and T_{d10} ranged from 278-419 °C. These TGA values suggest that all the synthesized PAAs and PIs are substantially stronger to resist thermal degradation up to high temperature of 200 °C. The aromatic region in their backbone structure, intermolecular forces and the amide linkages can be attributed to their high-thermal resistance.

The T_g values were measured by DSC under a nitrogen atmosphere and summarized in Table 3. The polymers synthesized show T_g values over 150 °C mainly, which were higher than those of conventional bio-based polymers. PIs show even higher T_g values mainly above 200 °C. The charge transfer from the electron rich bio-derived diamine to the electron deficient dianhydride and the high stability of the 5-membered imide ring due to the extended n-π conjugation between the carbonyl group and the lone pair of nitrogen atom could be the reasons for the high T_g values. Materials with high T_g values are suitable for application as super-engineering plastics. The mechanical properties such as tensile strength, Young's modulus, and elongation (summarized in Table 3) were determined by tensiometer at an elongation speed of 0.5 mm/min at room temperature.

Table 3. Thermal and mechanical properties of bio-based PAAs and PIs from 4APhe with different dianhydrides.

Polymers	T_{d5} [a] (°C)	T_{d10} [a] (°C)	T_g [b] (°C)	σ [c] (MPa)	E_{init} [c] (GPa)	ε [c] (%)
PAA-1	134	176	130	ND	ND	ND
PI-1	ND	ND	ND	ND	ND	ND
PAA-2	148	188	144	ND	ND	ND
PI-2	224	278	208	ND	ND	ND
PAA-3	228	290	210	44	2.5	1.0
PI-3	384	419	275	125	3.8	10.0
PAA-4	224	268	210	23	2.0	5.0
PI-4	368	394	258	110	15.0	4.5
PAA-5	190	207	175	ND	ND	ND
PI-5	255	280	243	86	3.5	4.2
PAA-6	180	209	175	ND	ND	ND
PI-6	248	378	225	84	2.9	2.9
PA 11 [d]	-	-	29	67	1.3	320
PET [d]	-	-	80	80	3.0	70
PLA [d]	-	-	60	80	3.5	6.0
PHA [d]	-	-	2	25	1.5	10
PC [d]	-	-	150	62	1.9	200
PMMA [d]	-	-	121	60	2.3	3.1

a) T_{d5} and T_{d10} refer to temperatures at 5 % and 10 % weight loss, respectively, measured using TGA at a heating rate of 5 °C/min under N_2. b) T_g refers to glass transition temperature, measured by DSC at a heating rate of 10 °C/min under N_2 atmosphere. c) Tensile strength at break, σ, Young's modulus, E_{init}, elongation at break, ε, and strain energy density, U, were estimated from stress-strain curves recorded using tensiometer at room temperature. ND means not determined due to brittleness. Values in parentheses are determined using film specimens. d) PA11, F-PI, PET, PHA, PLA, PC, and PMMA refer to poly(11-aminoundecanoic acid), fluorinated polyimide (from hexafluoroisopropylidene diphthalic anhydrides and 2,5-diamino-2,5-dideoxy-1,4: 3,6-dianhydrosorbitol), poly(ethylene terephthalate), polyhydroxyalkanoates, poly(lactic acid), poly(bisphenol A carbonate), and poly(methyl methacrylate), respectively.

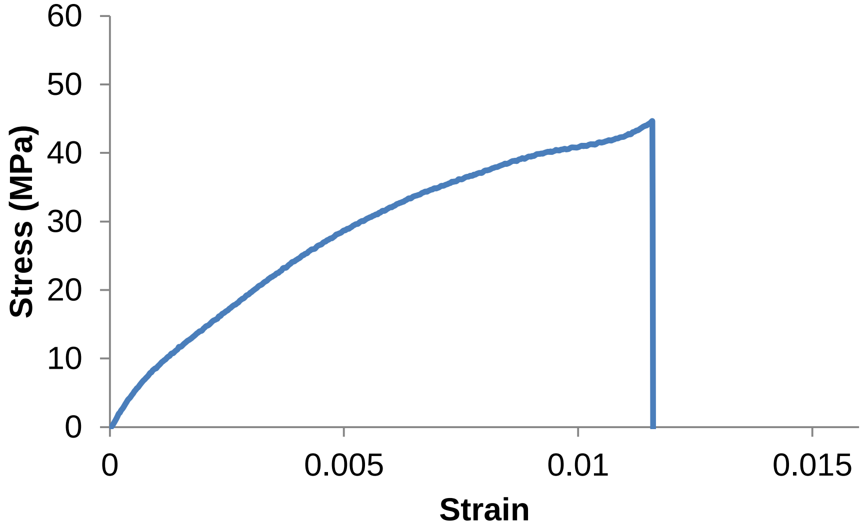


Figure 27: Stress-strain curve of representative PAA.

The films of PAAs and PIs showed tensile strength range from 45 to 100 MPa, Young's moduli of 2.0 to 5.0 GPa, and elongation at break of 1.10 % to 2.0 % respectively (representative curve in Figure). Additionally, the carbon fibre reinforcement (CFRP) with these polymers enhances their strength multifold as evident from mechanical properties in next chapter. The high tensile values indicate the impressive mechanical strength and excellent flexibilities of some of the PAAs and PIs.

3.4 Conclusion

We prepared various PAAs and PIs from exotic aromatic diamine, 4-aminophenylalanine (4-APhe) which we prepared from fermenting glucose using genetically manipulated *Escherichia coli* in reported shikimic pathway. 4-APhe was then polycondensed with a number of dianhydrides. The synthesized PAAs and PIs show good thermal resistance with T_{d10} values mainly above 250 °C, and T_g values mainly above 200 °C, improved tensile strength of 45 MPa, elongation at break at 1% or above, and a Young's modulus maintained around 2.5 GPa. In addition, the PAAs showed good adhesion with carbon fibres which further showed remarkable mechanical strength to be used for super-engineering plastics. The detailed properties are shown in next chapter. In general, the polymer materials produced using bio-source is strong enough to be used as high-performance materials, especially the combination of 4-APhe with BTDA and OPDA showed amazing results. I hope this little attempt of my research will further open new frontiers of developing high-performance polymeric materials based on exotic bio-sources.

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Chapter 4

Carbon fibre reinforced polymers (CFRP) using synthesized biopolymers in the present research and investigation of their mechanical properties.

Chapter 4

4.1 *Introduction*

The quest of a scientist can never be quenched! As a result of the extensive research on polymeric materials with numerous advantages, human beings live a comfortable life. But the discovery of new materials and improvement of existing one continued. One of the areas which gained so much recognition is development of carbon fiber reinforced polymer (CFRP). In very simple terminology, Carbon fiber reinforced polymer, carbon fiber reinforced plastic or carbon fiber reinforced thermoplastic (CFRP, CRP, or CFRTP), is an extremely strong and light fiber-reinforced plastic which contains carbon fibers (1, 2).

CFRPs can be expensive to produce but are commonly used wherever high strength-to-weight ratio and rigidity are required, such as aerospace, automotive, civil engineering, sports goods and an increasing number of other consumer and technical applications. The binding polymer is often a thermoset resin such as epoxy, but other thermoset or thermoplastic polymers, such as polyester, vinyl ester or nylon, are sometimes used (3-10). The composite may contain other fibers, such as an aramid (e.g. Kevlar), aluminium, ultra-high-molecular-weight polyethylene (UHMWPE) or glass fibers, as well as carbon fiber. The properties of the final CFRP product can also be affected by the type of additives introduced to the binding matrix (the resin). The most frequent additive is silica, but other additives such as rubber and carbon nanotubes can be used (11-13).

In 2011, Teijin built a 4 seater concept car. The body structure for this car was constructed with Teijin's thermoplastic CFRP (Carbon Fiber Reinforced Plastic), which weighs merely a fifth of a comparable steel structure. As carbon fibers have 10 times the strength yet a quarter of the weight when compared to steel, CFRP, a composite material made of carbon fibers and resins, has been used to date to reduce the weight of aircraft and other industrial materials such as wind generator blades.

Figure 1: Car made by Teijin Japan from CFRP material. Pic courtesy: Teijin website.

CFRPs are composite materials (14-17). In this case the composite consists of two parts: a matrix and reinforcement. In CFRP the reinforcement is carbon fiber, which provides the strength. The matrix is usually a polymer resin, such as epoxy, to bind the reinforcements together. Because CFRP consists of two distinct elements, the material properties depend on these two elements. The reinforcement will give the CFRP its strength and rigidity; measured by stress and elastic modulus respectively. Being a unidirectional material, the product can be significantly strengthened in certain areas with its layering design. This enables a completely different design than by using metal materials and opens up more possibilities that are beneficial for the item’s intended use.

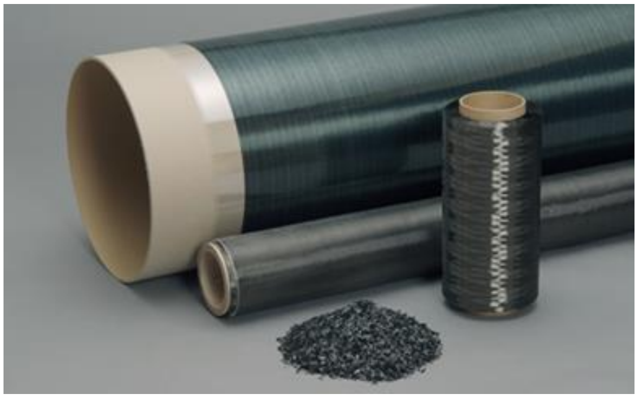


Figure 2: Carbon fibres in different forms such as thread role, fibers, sheet.

The development of CFRP materials is a fascinating theme for researchers' worldwide (18-23). The introduction of new products and technologies through various research activities, and the mass production through industries, and the business activities contribute to the development of society. However, the depletion of fossil fuels and increasing CO_2 emissions are major environmental problems in the world today. In response to such issues, the material development through research activities such as composites that contribute to weight savings in mobility, as well as recycling technologies, bio-based polymer materials and advanced materials.

Through our research activities, Kaneko group at JAIST will continue its ceaseless efforts, challenging to contribute to harmony with the environment and the sustainable growth of the society. With this objective, I have tried to develop CFRP materials using bio-polyureas and bio-polyimides polymer synthesized from microbial derived 4-aminophenylalanine as diamine monomer by incorporation in commercially available carbon fibres (19, 20), and investigate the mechanical properties of the composite materials. Though, there is great possibility of this research expansion to investigate or analyze other physical aspects such as testing electrical properties, corrosion resistant ability, chemical inertness, and fire resistance properties of these composite materials. However, in the time-constraint study, I could investigate few of the mechanical properties of CFRP materials developed at laboratory scale. Nevertheless, this work would garner enough attention for further research in the related field.

4.2 Experimentation

4.2.1Materials

The unsized PAN-based carbon fibres were purchased from TORAY KAKUROSU^{TM} (one of the renowned Japanese company known for carbon fibres composite materials). The matrix system used consisted of laboratory synthesized bioplastics, mainly polyureas and polyamic acids (precursor of polyimides), the syntheses of which have been described in previous chapters.

Figure 3: Carbon fibres from Toray Kakurosu^{TM}.

4.2.2 Preparation of CFRP sheets.

The carbon fibres were cut in a particular size. The sized carbon fibres were first heated at 100 °C for 15 min in the oven. Then, on a pre-heated silicon wafer or glass plate, the melt polymer (in dmf solvent) was casted on carbon-fibres to prepare composites. Then the composite materials were heated at 120 °C for 1 hour without pressure. Through this process, the solvent gets evaporated after proper mixing of polymeric substance with the carbon fibres. After this step, the composites were allowed to cool at room temperature. Typically, the content of the polymeric matrix was kept in the range of 30-40 wt. % concentration. For mechanical testing, different samples were cut in 30mm in length, 10 mm in width and the thickness was measured separately. The tensile measurements were carried out at an elongation speed of 0.5 mm/min on a tensiometer (Instron 3365, Kawasaki, Japan) at room temperature using polymer rectangle film with a length of 3 cm, a width of 1 cm and a thickness of 3 µm.

CRPF-PU-1 is polyurea (Me-4-APhe-MDI) based, CRPF-PU-21 is polyurea (Me-4-APhe-MMDI) based, CRPF-PU-3 is polyurea (Me-4-APhe-1,3PDI) based, CRPF-PU-4 is polyurea (Me-4-APhe-1,4PDI) based while CFRP-PAA-1 is polyamic acid (4APhe-CBDA) based, CFRP-PAA-2 is polyamic acid (4APhe-PMDA) based, CFRP-PAA-3 is polyamic acid (4APhe-BTDA) based, CFRP-PAA-4 is polyamic acid (4APhe-OPDA) based, CFRP-PAA-5 is polyamic acid (4APhe-BPDA) based, CFRP-PAA-6 is polyamic acid (4APhe-DSDA) based. Compared to polyurea precursors in CFRP materials, the polyamic acid based CFRP materials show higher mechanical strength presumable due to the rigid structure of polyamic acids which are further precursors of polyimides where 5-membered imide ring along with aromatic backbone is tough, and the interaction between inter-chain functional group is higher.

The results of mechanical testing is summarized in Table 1 followed by stress-strain curves for few samples.

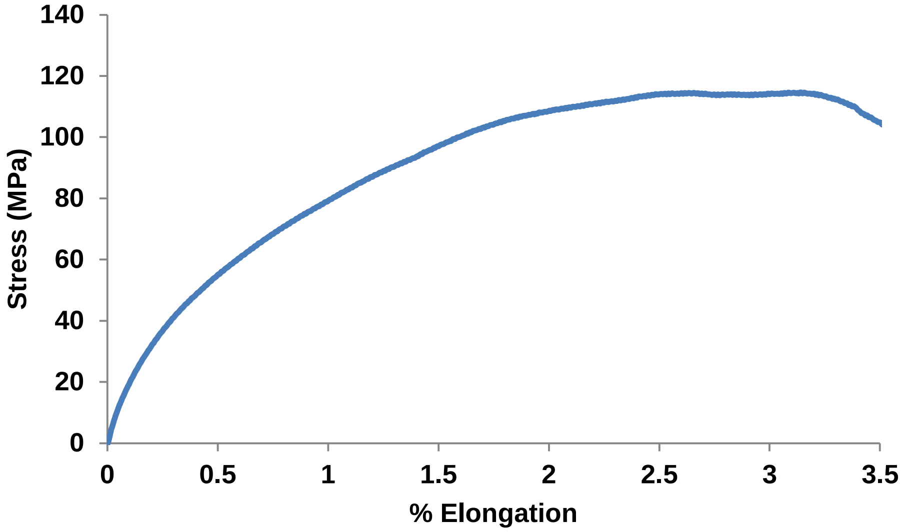
Table 1. Mechanical properties of CFRP materials from biobased polyureas and polyimides from 4-aminophenylalanine as one of the main monomer.

Polymers	σ [a] (MPa)	E_{init} [a] (GPa)	ε [a] (%)
CFRP-PU-1	45	4.2	1.2
CFRP-PU-2	14.1	2.1	2.5
CFRP-PU-3	79.3	7.9	3.2
CFRP-PU-4	61.6	6.1	3.1
CFRP-PAA-1	68.5	6.5	4.5
CFRP-PAA-2	ND	ND	ND
CFRP-PAA-3	114	8.4	2.5
CFRP-PAA-4	91	8.2	1.2
CFRP-PAA-5	ND	ND	ND
CFRP-PAA-6	60.5	5.9	2.7
Carbon fibres	47.8	4.9	3.4
HDPE [d]	37	1.3	320
PP	19.7-80	3.0	70
Pine wood (parallel to grain)	40	3.5	6.0

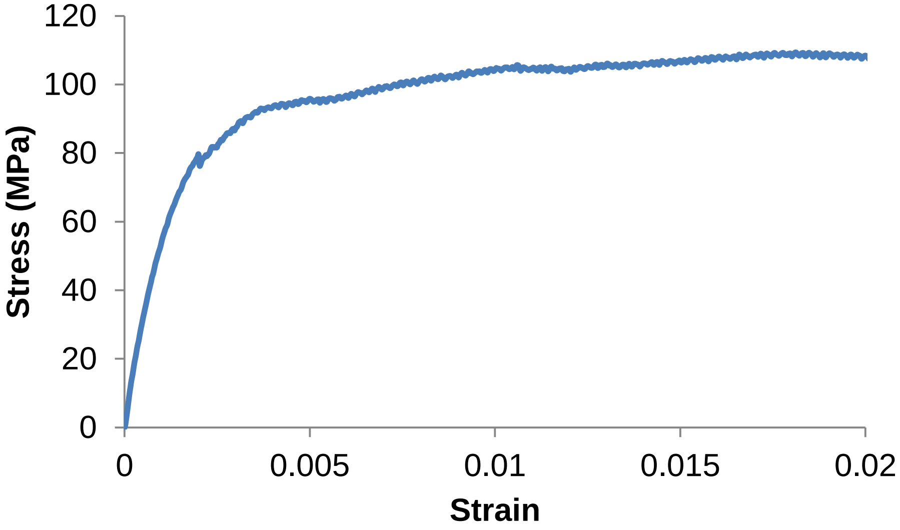
a). Tensile strength at break, σ, Young's modulus, E_{init}, and elongation at break, ε, were estimated from stress-strain curves recorded using tensiometer at room temperature. ND means not determined due to non-trial. b). HDPE and PP refer to High density polyethylene and Polypropylene respectively.

Some of the representative stress-strain curves for each sample of polymers are shown as:

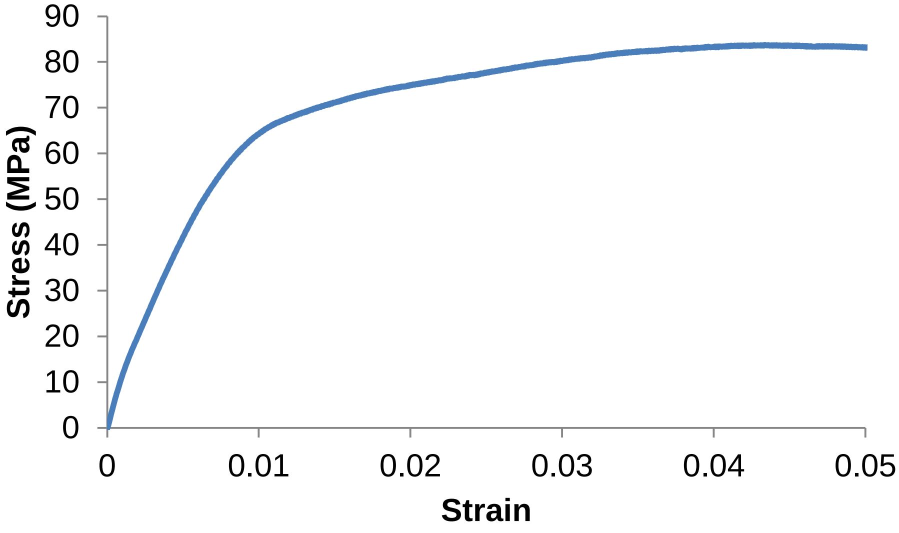
1. BTDA-PAA-CFRP (30mm, 10mm and 0.1 mm)



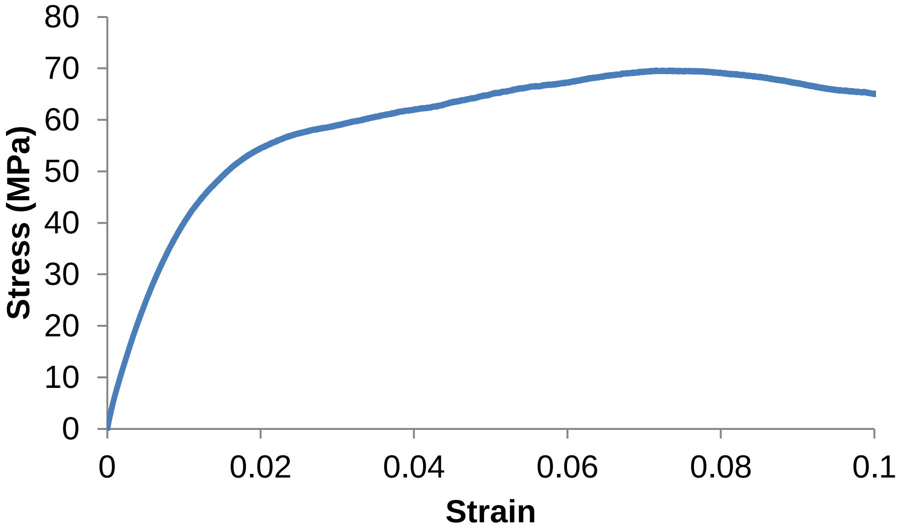
2. OPDA-PAA-CFRP: (30mm, 5mm, 0.042 mm):



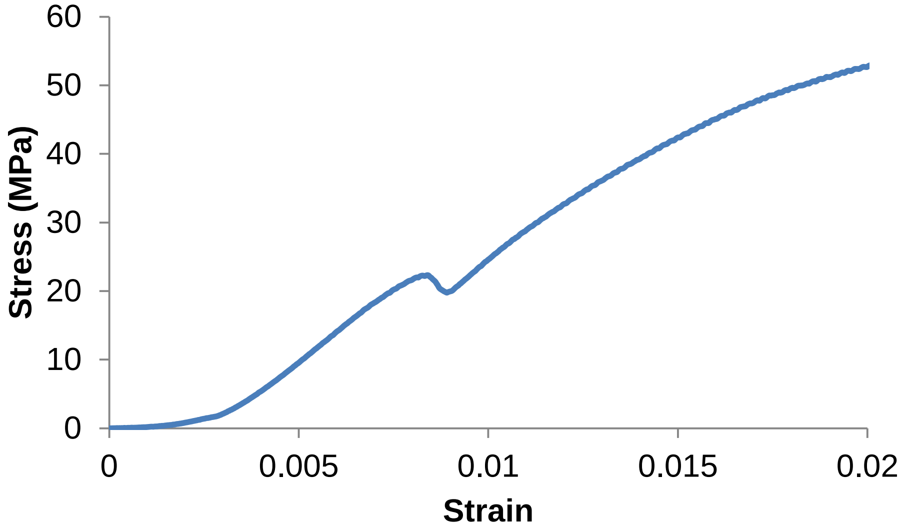
3. CBDA-PAA-CFRP (30mm, 10mm, 0.303mm):



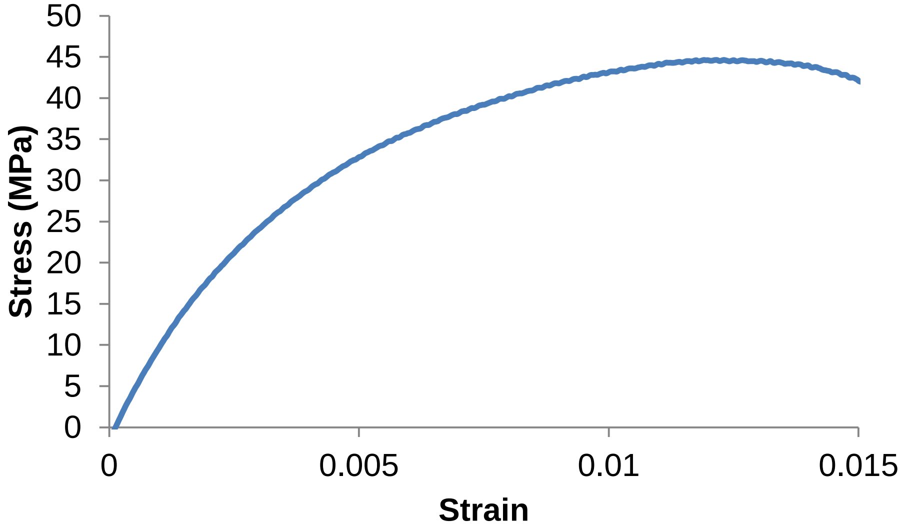
4. DSDA-CFRP-PAA (30mm, 10mm, 0.30mm)



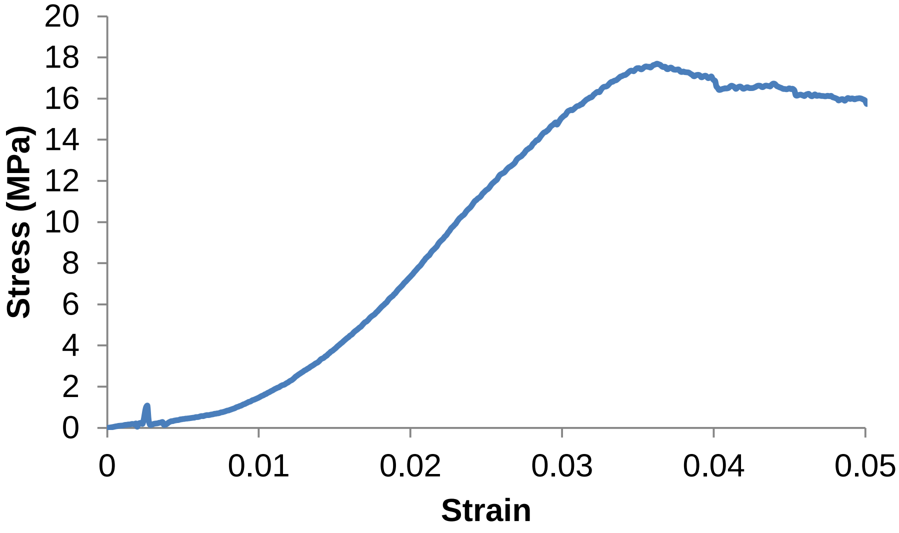
5. CARBON Fibres (30mm, 10mm, 0.207mm)



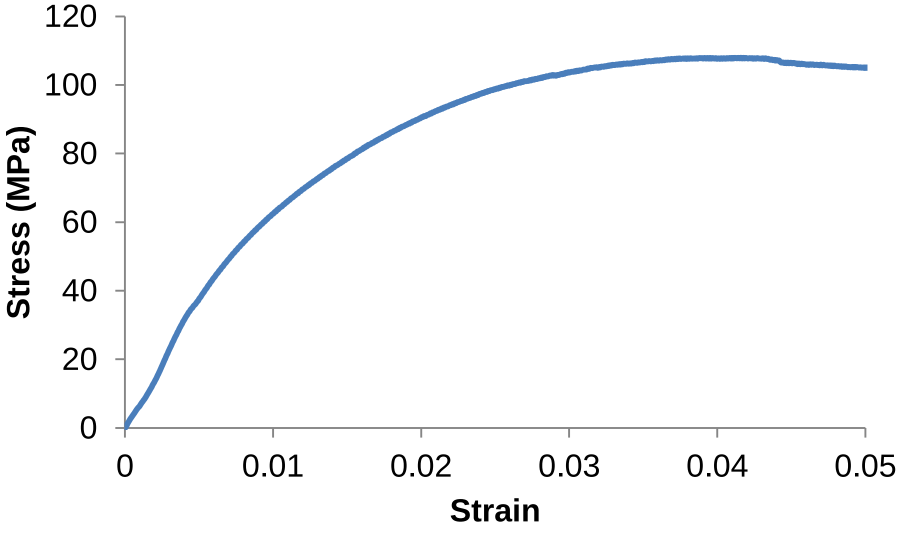
6. Me-4-APhe-MDI-CFRP (30mm, 10mm, 0.303mm)



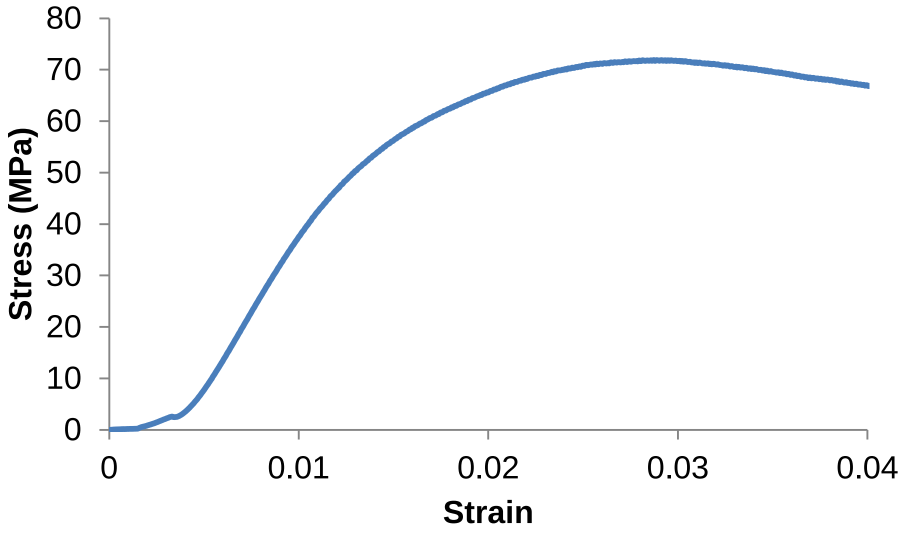
7. Me-4-APhe-MMDI-CFRP (10mm, 10mm, 0.303mm):



8. Me-4-APhe-1,3-PDI-CFRP (30mm, 10mm, 0.303mm)



9. Me-4-APhe-1,4-PDI-CFRP (30mm, 10mm, 0.303mm):



4.3 Discussion

The mechanical properties such as tensile strength, Young's modulus, and elongation at break were determined using synthesized CFRP composites and the carbon fibers in a tensile mode at room temperature (Table 1). We first compared the structural effects of carboxylate esterified polyureas matrix on the mechanical properties of composites formed using films which were more uniform compared to the randomly oriented carbon fibres. Further when the polymer matrix was changed from polyureas to polyamic acids, we found substantial increase in mechanical strength. Mechanical strength at maximum of polyurea based composites films ranged from 14 to 79 MPa while Young's moduli ranged 2.1-7.9 GPa, and elongation at break of 1.2-3.1 %. On the other hand, Mechanical strength at maximum of polyamic based composites films ranged from 61 to 114 MPa while Young's moduli ranged 5.9-8.4 GPa, and elongation at break of 1.2-4.5 %. The difference in mechanical strength of polyureas based composites and polyamic acid based composites can be explained by their basic structure. In the composite materials, two parts play an important role. One is matrix and other is reinforcement material. In our present study, we used carbon fibers as reinforcement material, which basically provides the strength. The

matrix is a polymer resin. Here we used polyurea and polyamic acid as matrix, which played an important role in binding the reinforcements together. Therefore, the material properties depend on these two materials. The combination makes the composites rigid and tough, which are measured in terms of stress and Young’s modulus. In earlier chapters, we have noticed the mechanical strength of polyamic acid or polyimides are higher than that of polyureas. Likewise, we obtain the results in composites. The more rigid backbone in case of polyimides due to aromatic ring and 5 membered imidazole ring contributes to the higher mechanical strength of polyamic acid or polyimides composites than polyureas composites which has flexible components too. However, the main challenge experienced in testing the mechanical strength of this composites material was complete break was not observed. The mechanical testing was therefore relied on the following factors, debonding between the carbon fiber and polymer matrix, fiber pull-out, and delamination between the CFRP sheet.
In order to compare the results with polyimide materials, further testing or improved methods are required.

4.4 Conclusion

In summary, a substantial improvement in the mechanical strength was shown by polymeric materials after reinforcement with carbon fibers owing to the increased Vander Waal forces and functional incorporation. Compared to the unsized carbon fibres, the composite materials formed with bioplastics show increase of tensile strength from 47.8 MPa to 114 MPa at maximum for the laboratory-sized carbon fibres. Although in this time-restraint study, I believe that more extensive research is required to substantiate the findings but nevertheless, it gives sufficient impetus to the researchers to develop carbon fibre reinforced plastics based on bioplastic matrix which give tough competition, or are equally competent with already established petro-based plastics.

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Chapter 5

Conclusive Remarks

Chapter 5

In chapter 1, the purpose of the study is defined along with few of the challenges faced. Further, the extension and the scope of the present research using the bio-available monomers are briefed as well. There is a well-known English proverb, “necessity is the mother of invention” which lays the foundation of scientific research for contribution to the society and welfare of mankind. The discoveries of new bio-based materials for polymers contribute to the development of sustainable green society, which is a ‘necessity’ in the present time owing to the growing dependence on plastics.

Bio-based polymers, especially, have attracted attention worldwide because of concern for establishment of a green sustainable low-carbon society, and their applicability in the development of broad spectrum of high-performance materials to meet specific needs. The thermomechanical performance of currently available bioplastics such as poly(lactic acid), poly(hydroxyalkanoate), and poly(butylene succinate) from bio-available aliphatic molecules are quite low which restrict their commercial or industrial usage. However, the introduction of aromatic rings in the backbone of polymer chain boosts their performance and enhances their applications in different spheres such as coating material as in electronic insulation, automobiles parts and transportation materials (14-18). Our research group has developed and reported aromatic bio-based polymers from hydroxycinnamate derivates such as *p*-coumaric acid (4-hydroxycinnamic acid), caffeic acid (3,4-dihydroxycinnamic acid) and ferulic acid (3-methoxy-4-hydroxycinnamic acid) for polyacrylate design. Our research further extends towards developing high-performance bio-based polyureas, polyamides, polyurethanes, and polyimides.

The background research or the overview of literature study, the goal of syntheses of bio-based polymers, the selection of appropriate monomers such as diisocyanates or dianhydrides with a newly proposed bio-available diamine, 4-aminophenylalanine (4-APhe) by our research group as the significant material for polymer syntheses, are the key features of chapter 1.

We present an unconventional and alternative source of polyurea syntheses by introducing a bio-based diamino acid, 4-aminophenylalanine (4-APhe) as one of the main constituents. Specifically the thermos-mechanical properties are evaluated for the synthesized polyureas or polyimides, and are compared with few of the conventional polymers. This newly proposed bio-derived aromatic diamine is a breakthrough for synthesizing large number of polymers. My findings in the research further propose this material quite competitive over conventional synthetic aromatic diamines for the syntheses of polymers.

Chapter 2 focuses on the design and development of polyureas using a bio-synthesized diamine, 4-aminophenylalanine (4-APhe) based on reaction with a series of diisocyanates such as MDI, TDI, PDI to mention a few. Some of the physical properties especially, thermal and mechanical properties along with some prospect applications of constructed polyureas are described.

Hence, these bio-based polyureas might potentially be used in sustainable plastics, coating materials, scaffolds for cell culturing, ophthalmological materials etc., and significantly improve the cost of products.

Chapter 3 mainly discusses synthesis of bio-derived polyimides (PIs) based on the reaction of microorganism derived aromatic diamine, 4-aminophenylalanine (4-APhe), with a series of dianhydrides. The thermal and mechanical properties of synthesized polyimides are also described. These polyimides have high thermomechanical properties, high transparency, and good cell-compatibility. Polyimides with 1,2,3,4-tetracarboxycyclobutane dianhydride (CBDA) as a counter monomer, are fully bio-based because CBDA is also a photodimer of biomolecular maleic acid derivates. Some of the characteristics of these polymers can lead to the development of high-performance and safe biomedical materials. The additional properties of excellent transparency and high refractive indices may lead to the development of ophthalmological materials.

Chapter 4, the incorporation of carbon fibres to the polymer matrix, and development of carbon fibre reinforced polymers (CFRP) materials was explained. Interestingly, the mechanical strength of the resulting material was high enough to be used for super-engineering plastics.

Chapter 5 is the concluding remarks of the present research based on the newly proposed bio derived monomer used in the present research. Some of the physical properties of the synthesized polymers are summarized in tables. I believe my research will significantly contribute to the polymer world through a novel proposal of bio-based monomer, and finally towards establishment of sustainable low-carbon society based on the synthesized bio-based polymers.

Future challenges and scope

The further challenges of this research lies in following ways.

1. Processibility: Extensive application of these bio-based plastics especially polyimides is still challenged by one or more of their possible inherent limitations, such as poor processability mainly because of their poor solubility in common solvents and high softening temperatures, and brittleness etc. The incorporation of additives such as plasticizers into the biopolymers might improve these inherent limitations. For general polyimides, bulky substituents can cause a significant increase in both T_g and the thermo-oxidative stability as well as increase the solubility of the polyimide, especially for polyimides with unsymmetrical or flexible groups in the backbone. Bulky and asymmetric substituents decrease the crystallinity and packing efficiency by distorting the backbone symmetry and restricting its segmental mobility. The extent of these effects depends on the number, size, and polarity of the substituents. These strategies generally suffer from a trade-off between the thermal properties and the solubility of a polyimide because the same structural features that enhance one characteristic will decrease the other. Therefore, to create more easily processed materials, a balance between these properties must be maintained without sacrificing the inherent high temperature resistance characteristics of these polyimides.

2. Blend materials: Blending these biodegradable polymers with metal ions or other fillers will greatly alter their physical and chemical properties, allowing modified properties to be used in new materials. The surface modification of polymers and metals play a predominant role in enhancing the surface energy for improved adhesion properties. For example: incorporation of metal salts such as TiO, ZrO modifies the polymer properties and makes them potential candidates for electronic applications for aerospace materials.
3. CFRP or carbon fiber reinforced plastic: Incorporation of carbon fibers to the polymer melt so as to make composite material, whose multifold enhanced thermos-mechanical properties make these materials a valuable asset for aircraft or spacecraft materials industry.
4. Cost of biopolymers. Apart from numerous advantages over synthetic counterparts, biopolymers face tough competition in terms of their cost. Improvement in processing or manufacturing techniques for monomers will ultimately reduce the polymer cost. The realization of cost-effectiveness of these materials must improve and they must contribute specifically to sustainable development.
5. Degradability: Techniques should be developed in order to fasten the process of their degradation. Some modification in the chemical structure of these polymeric materials especially polyimides might alter their solubility. Once soluble in common solvents, the degradation can be enhanced or controlled.
6. Bio-source: Polyimides which involves 1,2,3,4-tetracarboxycyclobutane dianhydride (CBDA) as a counter monomer, are fully bio-based because CBDA is also a photodimer of biomolecular maleic acid derivates. Further challenge lies in finding bio-source for other dianhydrides.

This work is my little endeavor towards development of high-performance polymers based on exotic bio based sources. I admit that there are lots of untapped portions which need to be researched. I just hope this work opens up a window in scientific community towards the use of biomaterials, thereby solving the most prominent *issues of the hour, which are, environmental concern and establishment of sustainable green society. The biomaterials are the need of the present and the future. We, as science students, have big responsibilities towards our mother earth. We can change the future of earth by enhancing clean living conditions for every organism just by switching from petro-based plastics to bio based plastics.*

Achievements

Below is the list of few achievements I got during my research tenure in the laboratory of Prof. Tatsuo Kaneko.

Conferences

International

Manjit Singh Grewal, Kazuya Taya, Seiji Tateyama, Tatsuo Kaneko***,** *Development of novel biopolyimides and their derivatives from functionalized aromatic amino acid*, IPC 2016, Fukuoka, registration no. 1475. *(poster)*

Manjit Singh Grewal, Kazuya Taya, Seiji Tateyama, Tatsuo Kaneko***,** *High performance biopolyureas and biopolyimides from 4-aminophenylalanine*, ICBP 2017, Taiwan, reference no. OB-113. *(oral)*

Manjit Singh Grewal, Kazuya Taya, Seiji Tateyama, Tatsuo Kaneko***,** *Carbon fibres reinforced polymer composites from amino acid-derived biopolyureas and biopolyimides*, ICBP 2017, Taiwan, reference no. PF-123. *(poster)*

Domestic

Manjit Singh Grewal, Kazuya Taya, Seiji Tateyama, Tatsuo Kaneko***,** *Unconventional alternative biopolyimides from exotic aromatic amino acid*, 66th SPSJ Annual Meeting – Makuhari Messe, 2017, id no. 101138. *(poster)*

Manjit Singh Grewal, Kazuya Taya, Seiji Tateyama, Tatsuo Kaneko***,** *Biopolyureas from exotic aromatic amino acid and their thermo-mechanical performance***,** 66th SPSJ Annual Meeting – Makuhari Messe, 2017, id no. 101139. *(oral)*

Manjit Singh Grewal, Kazuya Taya, Seiji Tateyama, Tatsuo Kaneko***,** *High-performance biopolyureas from unconventional exotic aromatic amino acid*, JAIST Japan-India Symposium on Materials Science 2017 *(poster)*

Publications

1. **Manjit Singh Grewal**, Kazuya Taya, Seiji Tateyama, Tatsuo Kaneko*, *Preparation of tough biopolyurea films from aromatic amino acid as diamine monomer*, *Macromolecular symposia*, accepted for publication on February 7, 2017.
2. **Manjit Singh Grewal**, Kazuya Taya, Seiji Tateyama, Tatsuo Kaneko*, *High performance bio-polyimides from 4-aminophenylalanine, an exotic aromatic amino acid*, (script in preparation).

Patent

A patent has been filed by our research group based on my polyureas research.

R&D exposure and others

1. Employed as a 'Researcher' in *R&D*, Green Science Materials Inc., Kumamoto (April 2016 ~ October 2016), a venture company of Prof. Tatsuo Kaneko and Dr. Okajima Kaneko.
2. Won '*Leave a nest'* Tech Plan contest, Kumamoto, July 2016.
3. Invited presentation at Tech Plan, Taiwan in August 2016.
4. Invited presentation at Minamata, Japan in October 2016.

Acknowledgement

Finally it’s time to move out and unveil the experience from a rollercoaster ride of PhD involving ups and downs, along with twisting and looping.

It is really hard to stay motivated during the journey. It is both painful and enjoyable experience as it involves lots of challenges, hardships, bitterness, frustration, encouragement, trust and kind support of many people as one climbs the ladder step by step. Implicitly or explicitly, this work bears on imprint of many people and it is really a pleasure for me to extend my debt of thanks with gratitude to all those who made this research work possible by making their support and kind help available in a number of ways.

First of all, my sincere gratitude goes for my supervisor **Prof. Tatsuo Kaneko** (Professor in Department of Advanced Science and Technology, Graduate School of Materials Science, JAIST). Without him, this journey would have not been possible. He is the man behind my dreams of PhD turning to reality. I have so much respect and love for him. He foreseen my caliber as a research scholar and showed me the road of PhD degree. He not only motivated me to pursue a PhD degree by accepting me in his laboratory but also held my hands firmly to keep me on the right track throughout the journey. He ingrained all those qualities in me which are required to transform a scholar into the potential scientist. He is also the person who is responsible for introducing me the world of polymers at depth. Under his supervision, I learnt to use very sophisticated techniques like Nuclear magnetic resonance (NMR, Bruker biospin AG 400 MHz) measurements, Fourier-transformed infrared (FT-IR) spectra, mass spectra using Fourier-transformed ion cyclotron resonance mass spectrometer (FT-ICR MS, Solarix), Thermogravimetry (TGA), TMA, differential scanning calorimetry (DSC), gel permeation chromatography (GPC), X-ray diffraction (XRD), Tensile and other measurements for polymers. He has patiently supervised, encouraged and guided me in the right direction all these years of PhD. Despite his busy schedules at times, he devoted some time to monitor my progress. He helped me in correcting presentations for various conferences and boosted my confidence for research. I would have not been able to complete the dissertation successfully without his constant support, guidance and kind help. He always helped me to organize the things beautifully and in a logical sequence to make the work readable and noticeable by his expert comments. He not only advised me how to improve manuscript but also suggested necessary readings. As a professor he knows my weak areas and has helped me improve them. He also aided me financially by employing me in projects like COI and MOE to cover some part of my expanses. He suggested me to work with collaborators, **Associate Prof. Kazuaki Matsumura** who is specialized in the field of “Biomaterials and cell culturing” for collaborative research which is also a part of PhD’s main work. I also thank Prof. Kaneko for giving me the opportunity to attend various seminars and conferences to explain my research at international level and meet new and interesting people.

I also wish to express my sincere gratitude to **Dr. Maiko Okajima Kaneko** for building the trust, confidence, courage, will, enthusiasm, motivation and curiosity in me. It has always been a pleasure to communicate and exchange ideas with her. The comprehensive discussions (sometimes in informal way) with her have helped me to shape up my knowledge. She is truly a genius. Her true devotion to research work has made me learn so much from her. Her praiseworthy remarks and constructive criticism have made me work harder. In spite of her busy schedule, she managed to get time for me for healthy discussions and innovative ideas. Without her spirit and role, this would have been merely an unaccomplished dream for me. She helped turning my PhD dreams into reality along with Prof. Kaneko. I have been extremely lucky to have a mentor who cared so much for her students. She has cared for my well-being and good health. She has always treated me like her own family member. I have immense respect for her. My words are not enough to show my gratitude to her. May she continue to contribute to the society through her valuable research and publications!

The research experience at Prof. Matsumura laboratory was very significant for me. Working with the team of intellectual and diligent members of Matsumura Sensei's laborattory gave me further insight into the research life and boosted my motivation to continue scientific research in future. There is so much to learn in the vast and dynamic field of science. I worked on L929 cell lines for my polymer films. L929 cells were so compatible with my polymer materials that the results obtained from cell culturing experiments substantiate the fact that our polymer films can be used for ophthalmological applications and other devices.

I thank Prof. Kazuaki Matsumura for providing me an opportunity to work in his research lab and for availing me all the possible facilities as and when required. Also he has thoroughly and convincingly guided me in the right direction, throughout our collaborative tenure. His handouts or reading material on the topic of "cell culturing" helped me building cell culturing concepts. Prof. Matsumura guided me in for mastering the art of cell culturing nicely and helped me in data analysis. He even praised me by saying that I was one of the few students with excellent skills in cell culturing in short time. It was really a wonderful experience working under his supervision. I extend my sincere regards to him.

Further, my deepest regards also extend to **Prof. Noriyoshi Matsumi** who has been my sub-supervisor. I appreciate his valuable advice and comments to improve my scripts and research proposals at times. Being from the next door neighbor laboratory, he has always monitored my journey and cheered me whenever he felt necessary. He even invited me to most of the seminars, symposium and lectures organized by him in the university. There is so much to learn from him as he is diligent, cheerful, intellectual, hard worker, excellent manager, tenacious, determined and charismatic with long vision.

Besides, I am also grateful to all the referees **Prof. Kohki Ebitani**, Associate Prof. **Takumi Yamaguchi**, Associate Prof. **Toshiaki Taniike**, and Prof. **Yoshiyuki Oishi** for their approval to be my referees in short notice, and their valuable contribution in my starting years of research at JAIST.

During my research I met **Prof. Shungo Kawanishi** whom I idolize, and have learnt numerous qualities of a social scientist which I have tried to incorporate in me. He is a great human being and is above Excellency. I have always looked up for him whenever I had faced any difficulty. He cheered me up during my moments of despair and hopelessness. His constant encouragement by discussion and never ending motivation has helped me to progress towards a better learner. He enlightened my way of thinking and helped to have broad vision. I don't have enough words for expressing my love, regards and respect for him.

My sincere thanks also extend to **all the lab members** of Prof. Kaneko. Some of them have already graduated and now working in renowned Japanese companies. Despite the language barrier, I have always been able to express and share a good camaraderie with them. Besides, we also discuss about culture, weather issues, food, and other stuffs. They all have been very motivating.

It's been my privilege to work in this institute (Japan Advanced Institute of Science and Technology) where I got many opportunities to study and grow as a stronger person in academic field and personal life. The university is well equipped with all the required research instruments. The infrastructure of the campus is serene and soothing for research. Here, I made many Japanese friends, and other international friends. I became accustomed to Japanese lifestyle because of their kind help. They even helped in learning the Japanese language, showing various sight-seeing places, and introducing good Japanese restaurants and culture here. I have also found a motherly figure in ***Emiko Matsuda San*** who has taken care of me like her own child. Besides I have visited various Japanese families who have brought great joy to my stay here in Japan.

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Back down the road there are others who have also encouraged me to pursue the road of PhD. **Ms. Rachna Narayanan**, my chemistry teacher during my high-school (Rishikul Vidyapeeth, Sonipat, Haryana, India), ingrained the seed of chemistry in me. She taught and helped me in building the concepts of the subject strongly. My **professors at Ramjas college** during graduation, and Mtech Dept. during post-graduation in Delhi University further enhanced my knowledge in the field. During PhD at JAIST, also there are many Professors who have taught various credit courses to me and helped me grow as a research scientist. I am thankful to all of them.

I also convey my heartfelt thanks to my institute Japan Advanced Institute of Science and Technology for financially supporting my research. Besides, this research was promoted by COI program "Construction of next-generation infrastructure using innovative materials" supported by JST. My heartfelt thanks to all the financial supporters.

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Pursuing a PhD degree is just like aspiring to reach a high peak. It is only after reaching the top of PhD ladder; I realize that the journey would not have been possible without the kind support of lots of people. It's really a proud moment to pen down sincere concerns and gratitude to all of the people mentioned above in this masterpiece.

I also pay my sincere tribute to two of my juniors from Delhi University, Mr. **Jatin Sharma** and Mr. **Yogesh Malhotra**, who met an unfortunate accident at Uchinada beach in Ishikawa, Japan on 14 July 2013, and lost their precious lives. Their sweet memories are always in my heart and I will treasure them ever forever. Their voices still echo in my ears and mind. I miss them. They are in my prayer every time.

On the top, I thank **Almighty** for letting me through all the difficult times. Without you, nothing would have been possible. I have felt your presence all the time. *Thank you God*.

Manjit Singh Grewal