

Synthesis of Novel Non-Isocyanate Polyhydroxyurethane from L-Lysine and its Application

Janine Booysen¹, Sanette Marx¹, LC Muller¹, Urban Vermeulen², and Anne Grobler²

Abstract— Plastic materials are traditionally produced from fossil-based chemicals and products and although recycling has lowered the amount of plastic material being deposited on land fill sites, the ecological impact of plastic materials such as polyurethane has been detrimental. Replacement of some of the fossil-based toxic components involved in the synthesis of polyurethane has the potential of making the material less hazardous and more biodegradable. In this study a novel approach for the synthesis of non-isocyanate polyurethanes (NIPUs) from bio-based amino acids were studied. Polycarbonates were used with different amino acids such as L-Lysine, L-Arginine, L-Glutamine and L-Asparagine to synthesise NIPUs and the performance of the amino acids were compared in terms of reactivity, source and structure. High density NIPU materials could be successfully prepared from only L-Lysine. Foamed NIPU materials were also prepared with the use of a blowing agent. The effectiveness of high density NIPU materials for use in diagnostic tests such as TB analysis was investigated by applying strips made of high density NIPU for capturing of ssDNA of *Mycobacterium tuberculosis* in a lysis microreactor.

Keywords— Amino acids, foams, *Mycobacterium tuberculosis*, non-isocyanate polyurethane, polycarbonate, polyhydroxyurethane, ssDNA.

I. INTRODUCTION

DUe to the toxicity of isocyanates used in conventional polyurethane production, new preparation methods are being sought [1]. The reaction of polycyclic carbonates and polyamines to form β -hydroxyurethanes with specific properties are a more safe and innocuous process for humans and the environment [2]. Five- and six-membered polycyclic carbonates can be used in the preparation of “green”, non-porous, moisture-insensitive polyurethanes which are free of isocyanate. Six-membered cyclical carbonates react more aggressively and smoothly with amines which results in higher polymerisation rates [3]. Hydroxyl groups that form at the β -atom of the urethane component increases the adhesion properties of the NIPU [2].

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Bio-based, renewable materials such as Soybean and linseed oil [4] and carbonates from bio-based polyols [3] have been investigated for the production of bio-based polyurethanes. The synthesis of polyurethanes requires a polyamine source which is traditionally isocyanate, but in the production of NIPUs the isocyanate can be replaced by suitable diamines [5, 6] amino acids [7].

The Preclinical Drug Development Platform (PCDDP) based on the North-West University's grounds has developed a new method for fast TB-diagnosis [8]. This method is identified by using extracted DNA from the bacteria. To increase the sensitivity of the test there is aimed to capture and concentrate the TB organisms DNA from a processed sample [9]. Replacement of the currently potential synthetic material strips with biodegradable materials can greatly reduce the impact of the waste from the diagnostics method.

In this project, NIPUs were synthesised using amino acids as polyamine source since amino acids can be extracted from plant materials such as *Amaranthus cruentus*. The effectiveness of different amino acids for the production of NIPUs from cyclic carbonate were compared based on their reactivity, structure and source. After successful synthesis, the NIPU materials obtained from L-Lysine were applied to the capturing of ssDNA in a lysis microreactor to assess its effectiveness as a scaffold for TB diagnostic tests.

II. METHODS

A. Materials

Poly (propylene oxide) bis-carbonate (PPO-Bis-C5, #BDG325) was used as the polyol in this experiment and was purchased from Specific Polymers (Castries, France). Three amino acids; L-Lysine (#BCBK4145V), L-Arginine (#MKBT0708V), L-Asparagine (#SLBK3555V), the diamine, 2,2'(Ethylenedioxy)bis(ethylamine) (Jeffamine EDR-148, #SHBD6238V), and the catalyst, 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD, #BCBP0084V), used in this study were purchased from Sigma Aldrich (Kempton Park, South Africa).

Poly (methylhydrogenosiloxane) Momentive MH15 (#S6343563 328) blowing agent and the other amino acid, L-Glutamine (#K44397389 510), were purchased from Merck Millipore (Modderfontein, South Africa).

B. Synthesis of Non-Isocyanate Polyurethane (NIPU)

The formulation of non-isocyanate polyurethanes were done according to methods described by Cornille and co-workers

[7]. One equivalent of the polycarbonate was mixed together with 0.05 equivalent of TBD compared to the polycarbonate. This mixture was vigorously stirred with a vortex for approximately 3 minutes in a glass vial or plastic cup. The amino acid/Jeffamine was then added to the homogenous mixture and mechanically stirred again for approximately 3 minutes with a vortex. The blowing agent (MH15) was subsequently added to the mixture to obtain foamed NIPUs. The mixture was then again stirred for roughly 2 minutes or until the mixture started to foam. The obtained polyurethanes were cured in an oven at 80°C for 12 hours and 120°C for 4 hours. Further drying of the material was done at 150°C for an additional 4 to 8 hours.

To increase the surface area for reaction and to improve mixing, the L-Lysine powder was crushed with a mortar and pestle until all of the lumps disappeared.

Another technique that presented a better outcome with more rigid polyurethanes was to mix the amino acid and polycarbonate-TBD mixture, in addition to the 3 minutes with the vortex, with a temperature controlled magnetically stirred hotplate at about 100°C and 1000 rpms. This mixture was stirred for roughly 2 hours or until the mixture started to turn solid. After mixing, the polyhydroxyurethane was cured at 80°C/120°C/150°C. The experimental formulations used in the synthesis of the high density NIPUs and polyurethane foams are given in Table I. The equivalents can be related from the amine hydrogen equivalent weight (AHEW) (g/eq).

TABLE I
NIPU FORMULATION IN EQUIVALENTS

Component	meq/g	1	2	3	4	5	6	7
PPO-Bis-C5	2.75	1	1	1	1	1	1	1
L-Lysine	13.68	1.05	-	-	-	-	3	1.05
L-Glutamine	13.69	-	1.05	-	-	-	-	-
L-Arginine	11.48	-	-	1.05	-	-	-	-
L-Asparagine	15.14	-	-	-	1.05	-	-	1.05
Jeffamine EDR-148	7.18	-	-	-	-	1.05	0.05	-
TBD	15.19	0.05	0.05	0.05	0.05	0.05	0.05	0.05
MH15		0.05	0.05	0.05	0.05	0.05	0.05	0.05

The reaction between the cyclic carbonate and amine can be seen in Fig. 1. The reaction scheme for the amino acid is very similar.

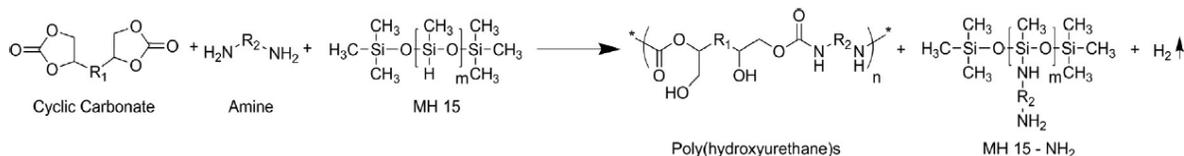


Fig. 1: General scheme for the formation of NIPU foams

Experimental results showed that only L-Lysine produced viable and stable high density polyurethane and polyurethane foam. Only the results obtained from using L-Lysine as replacement for amines in the synthesis of NIPUs will thus be discussed in this paper.

III. RESULT AND DISCUSSION

A. Non-Isocyanate Polyurethane Production

The novelty in preparing NIPUs from amino acids and polycarbonates necessitated a large number of experiments to obtain reagent proportions and operating conditions for the synthesis of viable polymers. The ratio of amine to polycarbonate, catalyst content, use of Jeffamine EDR-148, use of blowing agent, mixing and curing time/temperature, mixing method and four different amino acids were varied in order to study the possibility of preparing novel NIPUs from amino acids and polycarbonate (PPO-Bis-C₅).

The formulations for the synthesis was used as given in Table I, but after a few experiments it was concluded that the polycarbonate was still unreacted after 14 hours at 80°C/120°C (see Fig. 2).



Fig. 2: Unreacted NIPU after 14 hours of curing

The amount of amino acid was slowly increased in increments until a suitable rigid NIPU was formed. L-Lysine showed the best results out of the four amino acids (L-Lysine, L-Glutamine, L-Arginine and L-Asparagine), since L-Glutamine, L-Arginine and L-Asparagine became heterogeneous mixtures after mixing occurred.

The L-Glutamine, L-Arginine and L-Asparagine relations were also increased up until 3 equivalents compared to the polycarbonate, from which the results stayed the same.

The NIPUs obtained from L-Glutamine, L-Arginine and L-Asparagine could not be used for analysis since these mixtures did not harden enough to form a polymer. The polycarbonate and amino acid separated after 14 hours at 80°C/120°C. Fig. 3 shows the NIPU mixtures that were obtained from L-Glutamine, L-Arginine and L-Asparagine.

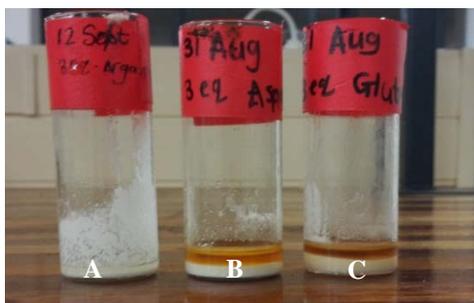


Fig. 3: NIPUs obtained from 3 equivalent L-Arginine (A), L-Asparagine (B) and L-Glutamine (C)

Three process operational changes were systematically made. The ratio of the amine towards the polycarbonate was increased, the mixture was placed in an oil bath to increase the reaction temperature and the mixing method was changed by stirring the mixture for a longer period at an elevated temperature. According to Blaine and co-workers [10], polycarbonate conversion increases with an increase in temperature because of the exothermic nature of the reaction. By allowing the NIPU mixture to mix more thoroughly with a temperature controlled magnetically stirred hotplate at approximately 1000 rpms and 100°C, the reagents were given more time to fully react with one another with the heat assisting in the reaction. The NIPUs with 2.5 and higher equivalent L-Lysine were the most rigid and thus the most viable for further analysis.

Jeffamine EDR-148 was similarly used in some experiments to obtain a reference of the structure and physical appearance of the NIPUs. A non-uniform liquid mixture was obtained though and thus this line of investigation was not further pursued (see Fig. 4).



Fig. 4: NIPU from Jeffamine EDR-148 and polycarbonate with MH15

It is believed that the structure of the L-Lysine and also its charge, positively influence the formation of NIPU. As stated by Blaine and co-workers [10], the reaction is temperature sensitive and by increasing the reaction temperature, the polycarbonate is more easily converted to NIPU. Since cyclic carbonates have a low reactivity, the use of a catalyst promotes the nucleophilic attack of the amine on the carbonyl group of the carbonate [10]. The reaction is therefore more prone to reach completion at higher reaction temperatures and extended reaction periods.

B. Physical Properties and Structure of Scaffolds

NIPU materials were successfully produced using L-Lysine and some of the polymers produced can be seen in Fig. 5. Fig. 5 also confirms the robustness and structure of the NIPU. Through the experimental procedures, it was understood that the reaction is influenced by both temperature and time. NIPU became more rigid with time and elevated temperatures. The synthesis of a NIPU foam was additionally investigated since NIPUs are more likely used in this form.

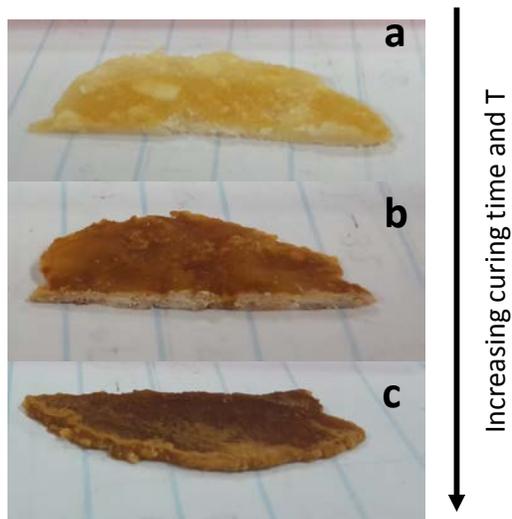


Fig. 5: NIPU materials produced from L-Lysine, cured for 8 hours at 80°C and 4 hours at 120°C (a), cured for an additional 4 hours at 150°C (b) and cured for an additional 8 hours at 150°C (c)

It was determined that the blowing agent reacts quicker with the polycarbonate, than the amino acid with the polycarbonate and no NIPU was therefore formed. By allowing the NIPU reaction to take place first before adding MH15, it was ensured that a NIPU material was created. It was further concluded that since no reaction takes place between the amino acid and MH15, Jeffamine EDR-148 was added to the mixture since it has a higher reactivity towards the blowing agent. By following the described method, a NIPU foam was successfully produced which could be handled and used for analysis. Properties of the foam can be improved by allowing the polycarbonate and amino acid to further react before introducing the blowing agent to the mixture. The equivalents that were used is tabulated in Table I. The obtained NIPU foam is shown in Fig. 6.

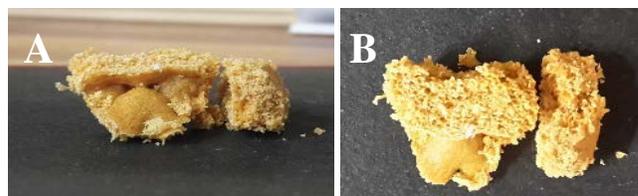


Fig. 6: A side (A) and top (B) view of NIPU foam produced from L-Lysine

Rigid NIPU scaffolds are needed to increase handling and observation. NIPUs that did not react completely showed definite separation and was still the golden colour of the

polycarbonate (Fig. 3). The best materials were obtained by using an excess of L-Lysine to allow the polycarbonate to fully react. These mediums were a light yellow colour and became whiter with more amino acids added to the mixture.

By placing the NIPUs made from excess L-Lysine (3 equivalent L-Lysine) for extra time in the oven, the materials became more rigid and turned a darker brown colour.

C. Characterisation of NIPU from L-Lysine

1) Scanning Electron Microscopy (SEM)

A NIPU foam was made from 3 equivalents of L-Lysine, 0.05 equivalent blowing agent (MH15) and Jeffamine EDR-148. The small amount of Jeffamine EDR-148 was added because it is more reactive than the amino acid and consequently forms H_2 gas which causes the material to become less dense and form a foam. The structure of this foam was studied using SEM imaging (see Fig. 7).

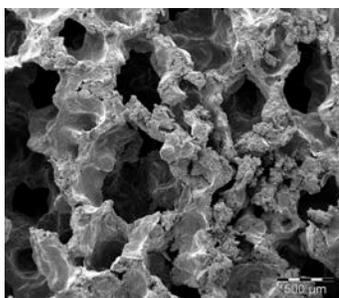


Fig. 7: SEM image of NIPU foam

Small holes can be seen with thick cell struts. The thickness of the cell struts is possibly reliant on the state of the mixture at which the blowing agent is introduced. If the MH15 is introduced to a liquid mixture, the cell struts will more likely be thinner and the cells will be larger or more homogeneous.

The structure and surface of the synthesized high density NIPU were also studied by SEM imaging and is shown in Fig. 8.

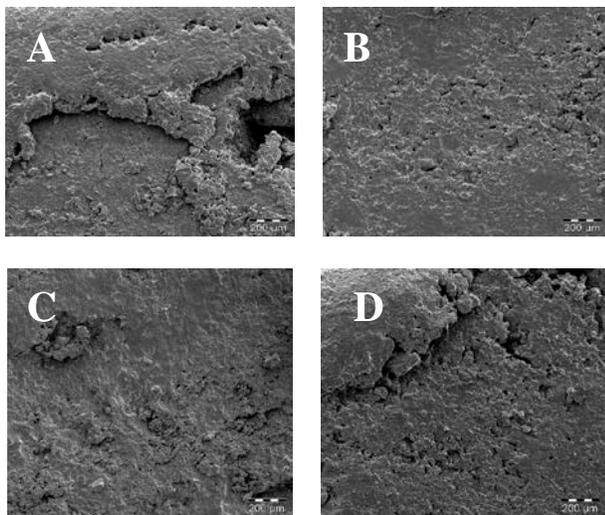


Fig. 8: SEM image of NIPUs synthesized using (A) 2.5 eq L-Lysine, (B) 2.75 eq L-Lysine, (C) 3 eq L-Lysine and (D) 3.25 eq L-Lysine

These materials showed very different surface properties to that of the foam. The surfaces of these materials were uniform and smooth which resulted in a more rigid material. The 3 equivalent L-Lysine medium showed small cracks of roughly

80 nm wide which were probably caused under high tension. Small granules visible is believed to be unreacted amino acids, which was in excess as previously discussed.

2) Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis was conducted on the solid NIPU materials synthesized using 2.5, 2.75, 3 and 3.25 equivalent L-Lysine compared to the polycarbonate mediums. A FT-IR spectrometer at 45 scans and 4 cm^{-1} resolution with a wavelength range of $4000\text{--}400\text{ cm}^{-1}$ was used for the analyses. FTIR analysis was also done on each one of the pure amino acids to serve as a reference for the NIPUs.

Various chemical bonds and functional groups in polyurethanes can be identified using FTIR analysis where different areas of absorption are associated with the different chemical structures. The spectra for pure L-Lysine is compared to that of two synthesized high density NIPUs in Fig. 9.

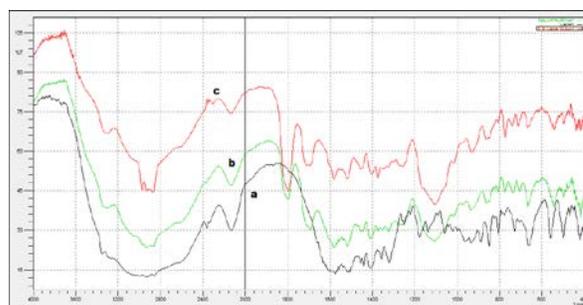


Fig. 9: FTIR spectra of pure L-Lysine (a), NIPU synthesised using 3 equivalent L-Lysine (b) and NIPU synthesised using 2.5 equivalent L-Lysine (c)

As seen from Fig.9, the pure L-Lysine does not have peaks at 1100 cm^{-1} , 1750 cm^{-1} and 1800 cm^{-1} , which are the C-O, saturated C=O and carbonyl groups that describe the polycarbonate. The material with the higher L-Lysine index shows smaller peaks at 1100 cm^{-1} and 1800 cm^{-1} which confirms that more polycarbonate is used during the reaction. The most prominent peaks in the spectra is listed and identified in Table II.

TABLE II
MAJOR PEAKS AND ASSIGNED CHEMICAL STRUCTURES

Peak position (cm^{-1})	Chemical structure	Reference
3300	-O-H stretching associated with free and bonded vibration	[11]
2980-2930	-C-H stretching of symmetric aliphatic-CHX	[12]
2880	-C-H stretching of asymmetric aliphatic-CHX	[13]
2100	Alkyne groups	[14]
1800	Saturated -C=O group in polycarbonate	[15]
1750	Carbonyl bonds	[16]
1560	Aromatic ring stretch	[16]
1480	-C-H stretching	[11]
1460	C-H deformation	[17]
1420	CH_2 bending	[18]
1370	Phenolic hydroxyl groups due to the polycarbonate	[18]
1260	-C-O ether vibration	[19]
1100	-C-O stretching	[17]
1020	C-O, C-C stretching or C-OH bending	[20]
940	Associated with polycyclic aromatic structures	[21]
780	Aromatic C-H vibrations	[11]

By curing the NIPU material for an extra 4 hours at 150°C, the C=O group in the polycarbonate at 1800cm⁻¹ started to disappear and the materials became more rigid and stable. By curing the material for an additional 8 hours at 150°C, the polycarbonate group became even less visible and the material more robust. It can thus be concluded that the reaction proceeds further and confirms that higher reaction temperatures are necessary to produce more rigid and stable materials. This comparison can be seen in Fig. 10.

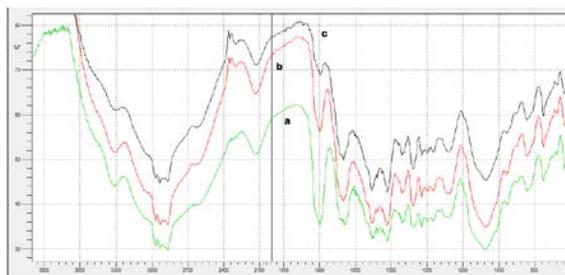


Fig. 10: FTIR spectra for NIPU materials after curing for 12 hours at 80°C and 4 hours at 120°C (a), curing for an extra 4 hours (b) and curing of the materials after an extra 8 hours at 150°C (c)

The most definite groups present in the polycarbonate is either C-H bond stretching or aldehyde C-H stretching with two bands at a wavelength of approximately 2960 cm⁻¹ and 2860 cm⁻¹. At 1800 cm⁻¹ the C=O group in the polycarbonate can be clearly seen along with the alkyne group (C=C) at 1670 cm⁻¹. The C-H groups are present between 1450 cm⁻¹ and 1300 cm⁻¹ with the acyl C-O groups identified between 1300 cm⁻¹ and 1260 cm⁻¹. The rest of the peaks at approximately 700 cm⁻¹ is due to typical alkene sp² C-H bends.

Another FTIR analysis was done with just the pure polycarbonate (PPO-Bis-C₅) to see whether the reaction reached completion and what type of groups are present in the polycarbonate. The FTIR spectra of pure polycarbonate, pure L-Lysine and a NIPU with 3 equivalents of L-Lysine is compared in Fig. 11.

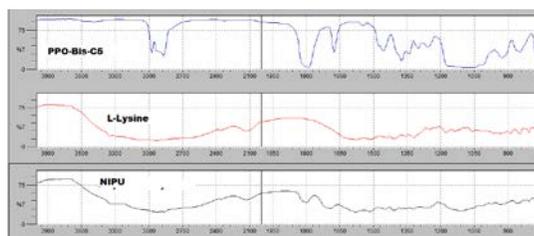


Fig. 11: FTIR analysis of PPO-Bis-C₅, L-Lysine and 3 equivalent L-Lysine

From Fig. 11 it can be concluded that the peaks/chemical structures that define the polycarbonate disappears after the reaction with L-Lysine occurs to form the NIPU. The polycarbonate therefore successfully reacts with the amino acid.

3) Thermal characterisation

To better understand the performance and structure of the NIPU, the thermal properties were investigated using TGA and DSC.

Differential scanning calorimetry (DSC) was used to attempt to determine the glass transition temperature of the NIPU materials. The thermal properties of the 11 mg sample was analysed on a Mettler Toledo DSC823^e instrument. The temperature range at which the analysis was done is between 30 and 200°C. The material was heated with a rate of 5°C/min under nitrogen atmosphere (50 ml/min). The DSC analysis graph is illustrated in Fig.12.

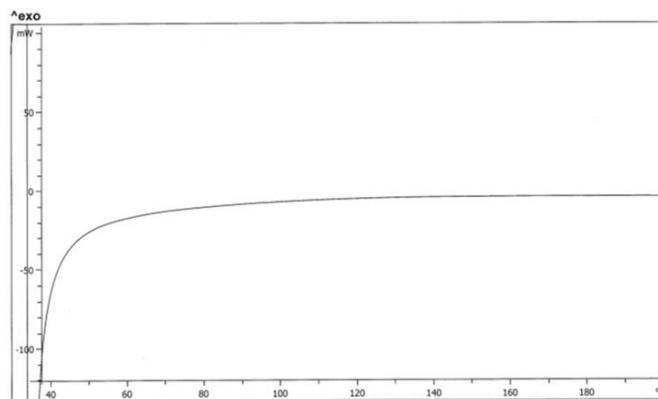


Fig. 12: DSC analysis graph

It can be seen from Fig. 12 that the glass transition temperature of the produced NIPU materials could not be observed from the DSC results. It was concluded from the analysis that the glass transition temperature is below 40°C which is consistent with respectable literature [7].

A thermal gravimetric analysis (TGA) was also conducted on the NIPU materials. A TGA analysis indicates the mass loss over a specific temperature range where decomposition of the material occurs. A mass loss of 91.67% was observed over a temperature range of 30-600°C. A Mettler Toledo TGA/SDTA851^e apparatus was used for the analysis with a heating rate of 10°C/min. The analysis was similarly done with a nitrogen purge (100 ml/min).

A three-stage degradation mechanism was presented by Saunders [22]. During the first degradation step, the isocyanate and alcohol is dissociated. The second step is when the primary amines and olefins are formed and the third degradation step consists of the formation of the secondary amines. According to Javni and associates, these three reactions may proceed similarly with NIPU [23]. The TGA curve is illustrated in Fig. 13.

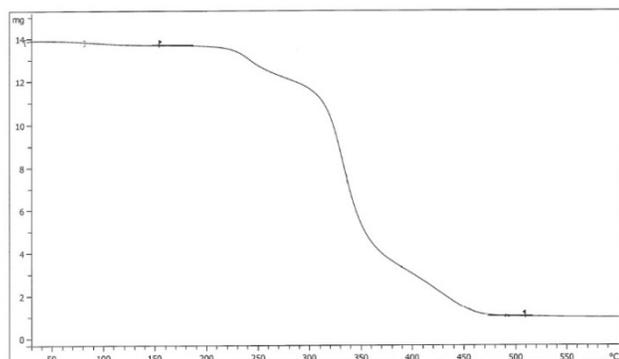


Fig. 13: TGA curve for NIPU

According to the TGA curve, the NIPU is thermally stable up until $\pm 200^{\circ}\text{C}$. The first degradation stage is roughly between 200 and 370°C with an 8% mass loss. This can typically be due to the degradation of urethane linkages to form CO , CO_2 and ammonia [15]. Hereafter, rapid degradation occurs between 300 and 365°C with a mass loss of approximately 9.6 mg. This corresponds to roughly 70% degradation. During the last stage, nearly 12.7 mg of the total NIPU material mass was lost. The degradation of the last stage occurs between 365 and 450°C .

D. DNA Immobilization

The ability of NIPU scaffolds have to capture DNA was tested by following the novel TB diagnosis method set out by the PCDDP. The scaffolds were firstly placed inside a lysis microreactor which operates at 95°C with the lysis buffer solution and non-infectious *Mycobacterium* cells. The mixture was mechanically stirred in the reactor at 3600 rpms for 7 minutes. The scaffolds were then removed from the lysis microreactor and transferred into a PCR cuvette. A PCR master mix was added to the cuvette and the cuvette was placed in a Philisa rapid thermocycler (Streck Inc., Omaha, NE). The 123 base pair DNA fragment size of the IS6110 gene target was amplified. The UV-transilluminated gel electrophoresis shows digital images of the PCR products [9]. A positive (+) and negative (-) control was used for comparison and to ensure that the process proceeded properly. A ladder (L) was used to identify the amplicon size.

Effective DNA immobilization was characterised with a bright band at the desired 123 base pair DNA amplicon value represented by the positive control. Six of the most rigid 3 equivalent L-Lysine NIPU scaffolds (see Fig. 14) were used for DNA immobilization tests.



Fig. 14: NIPU scaffolds used for DNA immobilisation tests

The transilluminated gel electrophoresis images of the amplification of the DNA captured by the scaffolds is shown in Fig. 15.

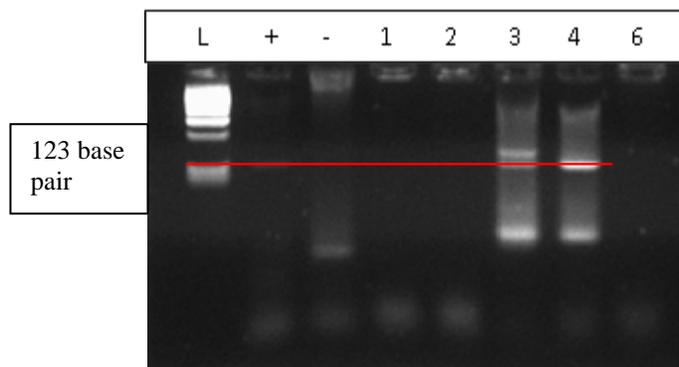


Fig. 15: Amplification of ssDNA captured by the scaffolds

Only one scaffold (scaffold 4) showed potential in capturing DNA compared to the positive control. Sample 3 has a dim band at the 123 base pair specific region but also a second unspecific band which is not ideal. Sample 5 could not be transferred effectively to the PCR due to the fact that it disintegrated too much in the lysis microreactor. All of the scaffolds became soft during the lysis process which made it difficult to transfer the samples to the PCR for the DNA capture process.

IV. CONCLUSION

It was shown that isocyanate can be successfully replaced with a renewable non-toxic compound in the synthesis of polyurethane. Non-isocyanate polyurethanes were synthesised from the reaction between a polycarbonate and amino acid. L-Lysine was the only amino acid that showed potential to replace isocyanate as diamine in the synthesis of polyurethane. Higher equivalent L-Lysine amounts compared to the polycarbonate resulted in more rigid and stable NIPU materials. By curing the materials for an additional period, the materials became more robust and stable and could undergo analyses. *Mycobacterium tuberculosis* single strand DNA could only be captured by one out of six NIPU scaffolds and therefore further development with regards to possible surface modification of the NIPU will be required for that application. The synthesis of NIPU materials with L-Lysine and a six-membered polycarbonate was thus successful.

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