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► **To cite this version:**

Roger Moussa, Samer Majdalani. Evaluating lateral flow in an experimental channel using the diffusive wave inverse problem. *Advances in Water Resources*, 2019, 127, pp.120-133. 10.1016/j.advwatres.2019.03.009 . hal-02419450

**HAL Id: hal-02419450**

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Submitted on 20 Dec 2021

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# Antibacterial Surface Based on New Epoxy- Amine Networks From Ionic Liquid Monomers

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## ABSTRACT

The design of new highly effective polymer materials as active surfaces or coatings against microorganisms such as *Escherichia coli* (*E. coli*) is a major challenge for public health. In the present work, novel imidazolium ionic liquid monomers (ILMs) having a similar structure of conventional Bisphenol A diglycidyl ether epoxy prepolymer (DGEBA) have been designed without requiring to the use of toxic and carcinogenic compounds *i.e.* Bisphenol A and epichlorohydrin. Then, a facile and efficient polyaddition reaction-based polymerization via a one step process was used in order to prepare unprecedented antibacterial epoxy-amine networks. The ultimate role of the monomers architecture on the epoxy conversion as well as on the reactivity of the epoxy-functionalized imidazolium ionic liquid monomers with an aliphatic amine (D-230) was investigated while the average molecular weight between crosslinks ( $M_c$ ) and the relaxation temperature of the resulting networks were determined. Thus, new epoxy thermosets with an excellent thermal stability, high hydrophobic behavior and good storage modulus at room temperature were produced. Finally, antimicrobial tests against *E. coli* were performed for the first time on these new cross-linked epoxy networks leading to a very strong inhibition of *E. coli* biofilm formation (- 95 %).

**KEYWORDS.** Ionic Liquids, Epoxy networks, Antibacterial activity, Thermo-mechanical properties, Epoxy conversion.

## INTRODUCTION

It is well-known that the presence of pathogenic microorganisms such as *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) are the origin of nosocomial infections causing real public health problems and generating high-

associated costs worldwide<sup>1-3</sup>. In order to decrease the spread of these microorganisms, various polymers acting as active surfaces or coatings have been widely used and reported in the literature to protect metal surfaces, soils, clinics walls and hospitals as well as in the field of surgery equipment, dental restoration, textiles, water treatment and food packaging<sup>4-8</sup>. Thus, various strategies were developed to obtain antibacterial activities as i) the use of nanoparticles, especially silver (Ag)<sup>9-12</sup>; ii) the synthesis of polymer brushes or layers<sup>13-15</sup>, iii) the incorporation of leaching antibacterial additives into polymer matrices<sup>16-17</sup> and iv) the synthesis of comonomers or polymers where the pendant groups act as antimicrobial agents<sup>18-22</sup>. Nevertheless, the majority of these different paths lead to other challenges to overcome such as the dispersion of the nanoparticles as well as their possible adverse effects on the human health, the complex polymer synthesis as well as a scale-up problem in the industry or the release of antibacterial groups in the nature thereby reducing their effect on the bacteria resistance<sup>22-23</sup>. Thus, the last approach seems the most promising to prepare surface-active antibacterial polymers.

Epoxy prepolymers are well-known to have good thermal, mechanical and adhesive properties as well as easy processing and low-cost production<sup>24-26</sup>. In recent years, various authors have developed bio- or epoxy networks with enhanced antibacterial properties<sup>27-29</sup>. For example, Liu *et al* have developed epoxy networks containing Tannic Acids that are well-known for their abundant terminal phenolic hydroxyl groups<sup>27</sup>. In fact, they have demonstrated that the use of high amounts of these phenolic groups resulted in enhanced antibacterial properties. Other authors have shown that the use of high quantities of eugenol bio-based epoxy prepolymers (between 30 and 70 wt %) induced significant reduction in bacterial adhesion ranging from 59 to 92 %<sup>28</sup>. According to the literature, quaternary ammonium salts are very effective agents against Gram-negative or Gram-positive bacteria and plays a key role on the antibacterial properties. In fact, the positive charge of the ammonium salts can generate a strong interaction with the

negative bacteria cell surface while the presence of a long alkyl chain can penetrate in the bacterial phospholipid membrane leading to a significant reduction of *E. coli*<sup>30-31</sup>. For these reasons, several authors have investigated on the insertion of ammonium salts or ammonium polymerizable groups in epoxy-amine networks<sup>32-34</sup>. Ritter *et al* have processed epoxy networks by using bisphenol A diglycidyl ether (DGEBA) and diethylene triamine (DETA) as co-monomers with the presence of different amounts of synthesized amino-ammonium products<sup>34</sup>. Thus, they have demonstrated that depending on the amount as well as the alkyl chain length of ammonium salts, epoxy-amine networks with good antibacterial properties could be developed. Alternatively, ionic liquids (ILs) are envisaged for distinct fields of research and applications<sup>35-37</sup>. It is mainly due to their unique properties such as chemical and thermal stabilities, low vapor pressure, antibacterial and antifungal properties and also versatility for tuning tailored combinations between cations and anions<sup>38-40</sup>. In particular, our research group has demonstrated that imidazolium and phosphonium ILs are useful platforms to design epoxy-IL networks with excellent thermomechanical properties, including thermal stability under nitrogen (> 300 °C), hydrophobic behavior and a glass transition temperature between 50 °C to 170 °C<sup>41-44</sup>. Very recently, a new reaction mechanism has been put forward for an epoxy-amine network derived from one imidazolium ionic liquid monomer bearing two epoxide functions<sup>45</sup>. Thus, the authors have shown that the use of an imidazolium ionic liquid monomer as co-monomer in the presence of a diamine could lead to the generation of quaternary ammonium species in addition to the three main reaction paths reported for the epoxy ring-opening reaction with a primary amine<sup>46</sup>. In this work, novel epoxy-amine networks were designed from three imidazolium ionic liquid monomers (ILM) and the impact of ILM architectures on the final properties of these epoxy networks were investigated. Thus, the reactivity of the imidazolium ionic liquid monomers with an aliphatic amine, denoted Jeffamine D230, was firstly investigated. Then, the morphologies as

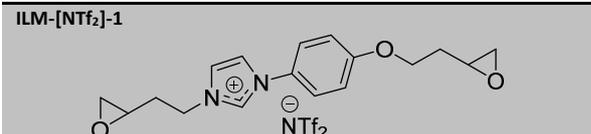
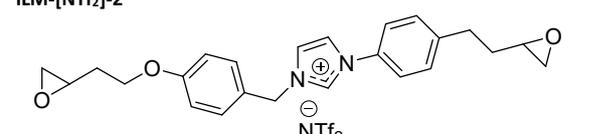
well as the thermo-mechanical behavior and the thermal stability properties were also studied. Finally, the potential of these unprecedented epoxy-amine networks containing 25 to 30 wt % of quaternary ammonium groups covalently linked was evaluated for the first time as new polymer materials with antibacterial properties.

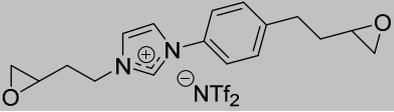
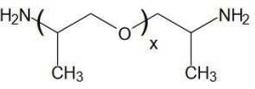
## EXPERIMENTAL

### Materials

All reagents were purchased from Sigma Aldrich, Alfa Aesar or TCI and were used without further purification and used as received. Solvents were used in RPE grade without further purification. Anhydrous solvents were obtained from a PURESOLV SPS400 apparatus developed by Innovative Technology Inc. In this work, all structures and properties of the materials used are summarized in **Table 1**. Epoxy networks based on imidazolium ionic liquid monomers denoted ILM-[NTf<sub>2</sub>]-1, ILM-[NTf<sub>2</sub>]-2, ILM-[NTf<sub>2</sub>]-3, liquid at room temperature, was synthesized according to the procedure described below. One commercial polyetheramine denoted Jeffamine D230 purchased by Huntsman was investigated as conventional hardener. The diamine was added in the stoichiometric ratio *i.e.* epoxy to amine hydrogen groups equal to 1, in pure mixture.

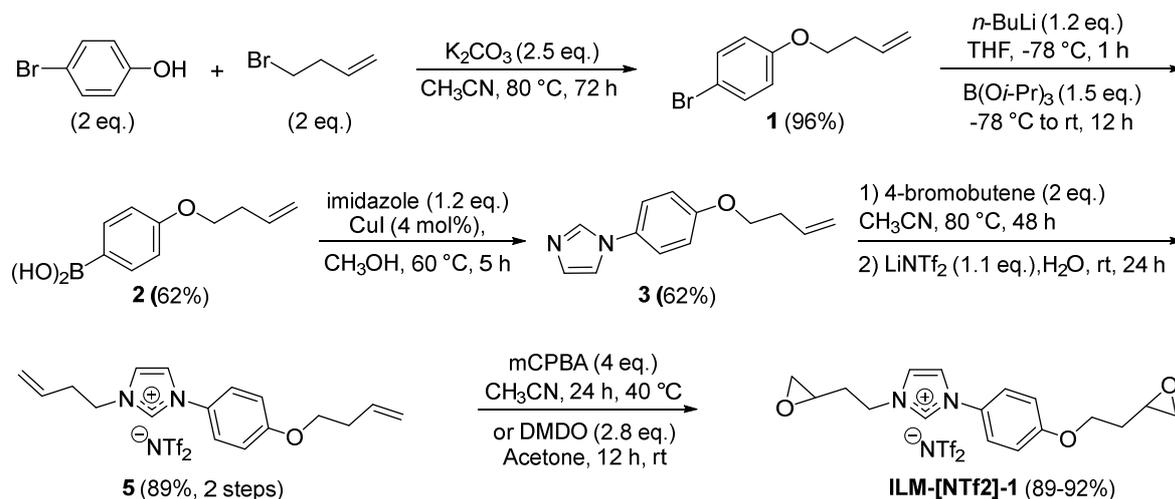
**Table 1.** Chemical structures, nomenclatures and properties of the materials used to design epoxy-amine networks

Chemical structure and name	Supplier	Properties
 <p>ILM-[NTf<sub>2</sub>]-1</p>	« Homemade »	Epoxy equivalent (EEW): 290.75 g eq <sup>-1</sup> T <sub>g</sub> = -46 °C
 <p>ILM-[NTf<sub>2</sub>]-2</p>	« Homemade »	Epoxy equivalent (EEW): 335.81 g eq <sup>-1</sup> T <sub>g</sub> = -26 °C

<b>ILM-[NTf<sub>2</sub>]-3</b> 	« Homemade »	Epoxy equivalent (EEW): 282.75 g eq <sup>-1</sup> T <sub>g</sub> = -46 °C
<b>Jeffamine D230</b> 	Huntsmann	Amine hydrogen equivalent (AHEW): 60 g eq <sup>-1</sup>

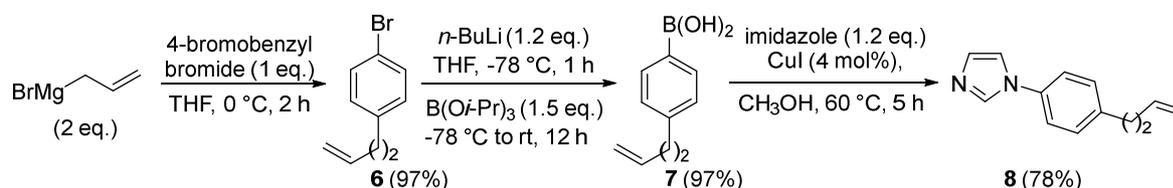
### Synthesis of ionic liquid monomers (ILMs)

Here, three imidazolium salts denoted ILM-[NTf<sub>2</sub>]-1, ILM-[NTf<sub>2</sub>]-2 and ILM-[NTf<sub>2</sub>]-3 were synthesized and analyzed by <sup>1</sup>H-, <sup>13</sup>C-, <sup>19</sup>F-NMR, infrared, and High-Resolution Mass Spectra. The first salt ILM-[NTf<sub>2</sub>]-1 was prepared according to our previous work from 4-bromophenol, 4-bromobutene, bis(trifluoromethane)sulfonimide lithium salt and DMDO or mCPBA as main reagents<sup>47</sup>. The six-step sequence to synthesize ILM-[NTf<sub>2</sub>]-1 is fully illustrated below (**Scheme 1**) and the NMR assignments of resonance peaks are reported in the supporting information (see **Part II and V, Figure S1-S6**).



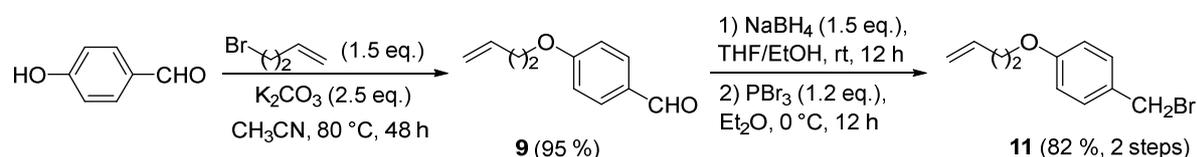
**Scheme 1.** Procedure synthesis of ILM-[NTf<sub>2</sub>]-1

The preparation of monomers ILM-[NTf<sub>2</sub>]-2 (**Part III** in the ESI) and ILM-[NTf<sub>2</sub>]-3 (**Part IV** in the ESI) requires a three-step sequence to synthesize imidazole **8** (**Scheme 2**). In the first step, the reaction involves 4-bromobenzyl bromide (1 eq.) and allylmagnesium bromide (2 eq.) as Grignard reagent to form compound **6** in excellent yield. The aryl bromide **6** was then converted to boronic acid **7** to promote a copper-catalyzed Chan-Lam coupling with imidazole (1.2 eq.). The heterocycle **8** was prepared in 78% yield after 5 h at 60 °C. This reagent was synthesized on a several grams scale using a reproducible sequence (**Figure S7-S9** in the ESI) will allow efficient access to both salts (ILM-[NTf<sub>2</sub>]-2 and ILM-[NTf<sub>2</sub>]-3).



**Scheme 2.** Procedure synthesis of the compound **8**

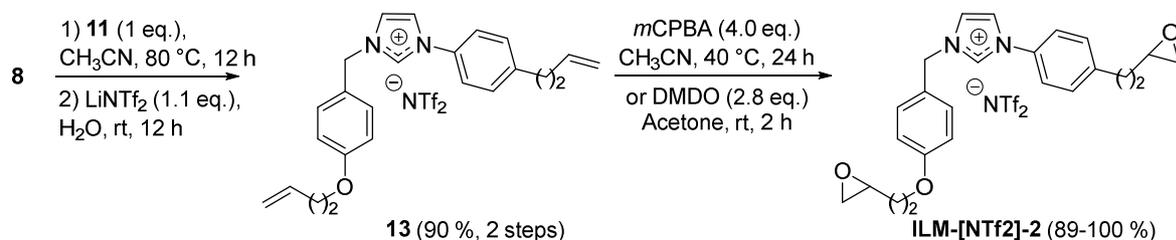
Thus, we optimized the synthesis of ILM-[NTf<sub>2</sub>]-2 bearing two aromatic rings and terminal epoxides. For this purpose, the alcohol function of commercially available 4-hydroxybenzaldehyde was alkylated by 4-bromo-1-butene (1.5 eq.). In the presence of potassium carbonate, the ether **9** was isolated in 95 % yield. The aldehyde function was reduced to alcohol **10** by sodium borohydride then subjected to bromination with phosphorus tribromide to give compound **11** in 82% yield overall yield (**Scheme 3** and **Figure S10-S12** in the ESI).



**Scheme 3.** Procedure synthesis of the compound **11**

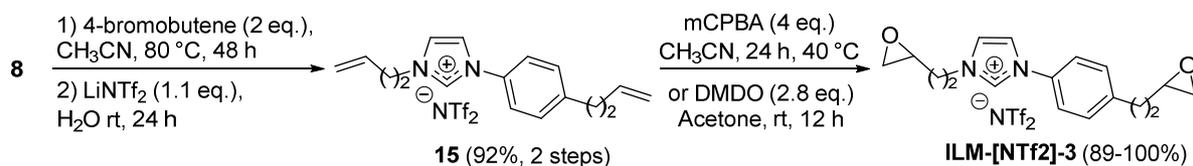
The synthesis of imidazolium NTf<sub>2</sub> **13** involves a nucleophilic substitution of benzyl bromide **11** with imidazole **8** and an anionic metathesis step with LiNTf<sub>2</sub>. Finally, the oxidation of the alkenes

was carried out in presence of an excess of mCPBA (4 eq.) at 40°C or DMDO (2.8 eq.) at room temperature. The overall yield of this sequence is **90 %** to afford ILM-[NTf<sub>2</sub>]-2 as clean oil (**Scheme 4** and **Figure S13-S15** in the ESI).



**Scheme 4.** Procedure synthesis of the ILM-[NTf<sub>2</sub>]-2 from compounds **8** and **11**

In order to study the behavior and the efficacy of the previous salts, we prepared ILM-[NTf<sub>2</sub>]-3 which was prepared from imidazole **8**. This salt was synthesized by quaternarization of the imidazole by 4-bromobutene (2 eq.) followed by the anion exchange with LiNTf<sub>2</sub> and oxidation under same conditions as previously. Once again, excellent yield was observed after this three-step sequence (**Scheme 5** and **Figure S13-S18** in the ESI).



**Scheme 5.** Procedure synthesis of the ILM-[NTf<sub>2</sub>]-2 from compound **8**

### Epoxy network processing

To prepare the network, ILM-[NTf<sub>2</sub>]-1, ILM-[NTf<sub>2</sub>]-2, ILM-[NTf<sub>2</sub>]-3 and Jeffamine D230 were mixed with a suitable stoichiometric ratio under stirring at room temperature during 30-40 minutes. The mixture was then degassed in an ultrasonic bath during 15-20 minutes and was

poured into silicone molds. Finally, epoxy-amine networks named ILM-1, ILM-2 and ILM-3 were cured 2 h at 80 °C and 3h at 120 °C.

### **Characterization methods**

*Thermogravimetric Analyses (TGA)* of ionic liquid monomers and the resulting epoxy networks were performed on a Q500 thermogravimetric analyzer (TA instruments). The samples were heated from 30 to 700 °C at a rate of 20 K min<sup>-1</sup> under nitrogen flow.

*Differential Scanning Calorimetry measurements (DSC)* of epoxy/IL blends and networks were performed on a Q20 (TA instruments) from 20 to 250 °C. The samples were kept for 3 min at 250 °C to erase the thermal history before being heated or cooled at a rate of 10 K min<sup>-1</sup> under nitrogen flow of 50 mL min<sup>-1</sup>.

*Surface energy of epoxy network* was determined with the sessile drop method using a GBX goniometer. From contact angle measurements performed with water and diiodomethane as probe liquids on the samples, polar and dispersive components of surface energy were determined using Owens-Wendt theory<sup>48</sup>.

*Fourier Transform Infrared absorption spectra (FTIR)* were recorded on a Thermo Scientific Nicolet iS10 Spectrometer with a transmission accessory from 4000 cm<sup>-1</sup> to 500 cm<sup>-1</sup> (32 scans, resolution 4 cm<sup>-1</sup>). The IR absorptions were observed as strong bands and are given in cm<sup>-1</sup>.

*Dynamic mechanical analysis (DMA)* was performed on rectangular samples with dimensions 15 mm and 5 mm and a thickness of 1 mm using an ARES-G2 rheometer with torsional fixture (TA Instruments). The material response was measured with a heating rate of 3 °C.min<sup>-1</sup>. All tests were performed within the linear viscoelastic region of each material at a frequency of 1 Hz. Storage modulus E', Loss Modulus E'' and the loss factor tan δ were measured during temperature ramps from -80 °C up to 150 °C.

*Transmission electron microscopy (TEM)* was performed at the Technical Center of Microstructures (University of Lyon) using a Phillips CM 120 microscope operating at 80 kV to characterize the dispersion of thermoplastic phases in the epoxy networks. 60-nm-thick ultrathin sections of samples were obtained using an ultramicrotome equipped with a diamond knife and were then set on copper grids.

*Liquid NMR*  $^1\text{H}$ -,  $^{13}\text{C}$ -, and  $^{19}\text{F}$ -NMR spectra were recorded on a Bruker Avance III 400 MHz, 500 MHz or AvanceNEO 600 MHz spectrometer. Samples were dissolved in an appropriate deuterated solvent ( $\text{CDCl}_3$ ). The chemical shifts ( $\delta$ ) are expressed in ppm relative to internal tetramethylsilane for  $^1\text{H}$  and  $^{13}\text{C}$  nuclei, and coupling constants are indicated in Hz. Abbreviations for signal coupling are as follows: s=singlet; d=doublet; dd=doublet of doublets; t=triplet; q=quartet; quin=quintet; m=multiplet; br=broad signal. To assign the signals to the different proton and carbon atoms, as well as the relative stereochemistry of the cycloadducts, additional 2D NMR experiments (COSY, TOCSY, HSQC, HMBC) and NOESY experiments were performed.

*Antibacterial activity* was investigated by *Escherichia coli* (DH5 $\alpha$ ) biofilm formation over the epoxy-amine polymers. A single bacteria colony was incubated overnight in Luria Bertani broth (LB, containing 10 g.L $^{-1}$  of peptone and NaCl, and 5 g.L $^{-1}$  of yeast extract) and then purified by washing with 1% NaCl twice and finally the pellet was suspended in phosphate buffered saline (PBS) solution. The optical density at 600nm (OD600) was determined and a solution of 0.1 OD was prepared in LB. The samples were cut into 4 mm squares, transferred to a 96-well plate containing 100  $\mu\text{L}$  of the last bacteria solution and incubated at room temperature. After 24h, the squares were gently washed with PBS and incubated with 10% AlamarBlue at 37  $^\circ\text{C}$  for 2h. The fluorescence indicating microorganisms' viability was then measured in a Enspire Multimode Plate Reader (Perkin Elmer) with excitation at 570 nm and emission at 585 nm. Biofilm

inhibition was then calculated considering the reference polymer denoted DGEBA/D230 signal as 100% viability (zero inhibition). All the experiments were performed in octuplicate.

*Scanning electronic microscopy (SEM)* was performed with a high-resolution FEI Quanta 650 FEG operating at 5 kV using secondary electron detectors. The samples of the epoxy-amine networks were fixed with 4% glutaraldehyde overnight at room temperature and dehydrated with ascending ethanol concentration solutions (25%, 50%, 75% and 100%) for 1h each. After ethanol removal, samples were kept in a desiccator overnight. Finally the squares were then mounted in aluminum stubs and sputter-coated with carbon.

## RESULTS AND DISCUSSIONS

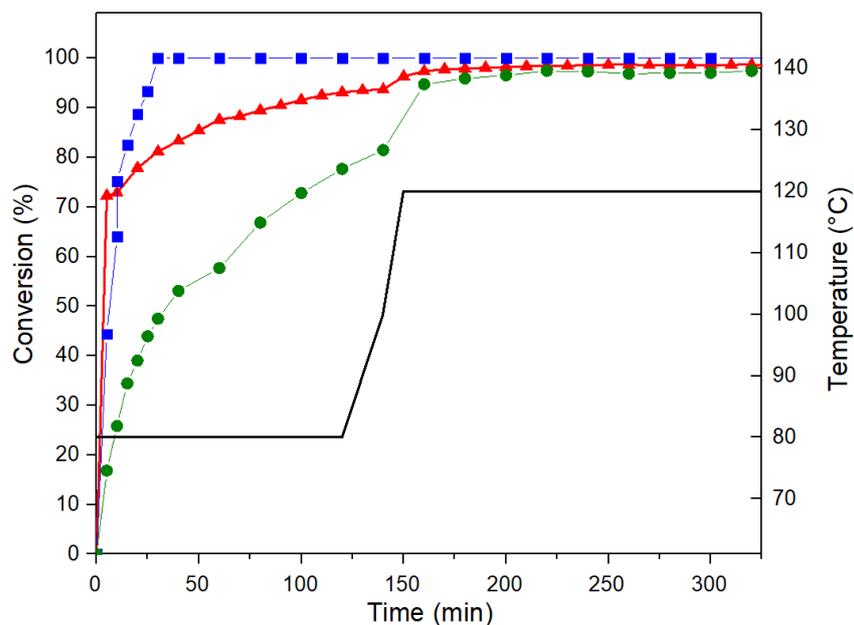
### Evaluation of the epoxy conversion (FTIR) and reactivity study (DSC) of epoxy-amine networks

The curing process as well as the determination of the conversion percentage of epoxide groups of all the systems was studied in the same conditions, *i.e.* 2h at 80 °C and 3h at 120 °C. Based on the literature, the evolution of the adsorption bands at 914 cm<sup>-1</sup> corresponding to epoxide groups (  ) and at 1184 cm<sup>-1</sup> which corresponds to the ether linkage (C-O-C) was followed by FTIR spectroscopy<sup>42,44</sup>. Taking this into account, the conversion of epoxide group was determined by using Equation (1):

$$X \% = \frac{A_0 - A_t}{A_t} \times 100\% \quad \text{Equation (1)}$$

where  $A_0$  and  $A_t$  are the ratio between the area of two absorption peaks (914 cm<sup>-1</sup> and 1184 cm<sup>-1</sup>) at  $t=0$  as well as the reaction time  $t$ . Thus, the conversion of epoxy groups versus curing time of

the three selected epoxy-amine systems, denoted ILM-1, ILM-2 and ILM-3, are summarized in **Figure 1**. In addition, the FTIR spectra of ILM-1, ILM-2 and ILM-3 during curing process are presented in the **Figure S19, S20, S21** on the supporting information



**Figure 1.** Epoxy group conversion as a function of curing time as determined by FTIR spectra for (●) ILM-1, (■) ILM-2, and (▲) ILM-3

An epoxy conversion higher than 96 % was observed for all epoxy-amine networks prepared with ILM-[NTf<sub>2</sub>]-1,-2,-3 confirming the good reactivity between these salts and Jeffamine D230 used as curing agent. However, different behaviors were observed depending on the imidazolium ILM-[NTf<sub>2</sub>] used. In fact, a very fast consumption of epoxy functions was observed for ILM-[NTf<sub>2</sub>]-2 (>90 % after 25 minutes) whereas a progressive conversion of these functions was detected for ILM-[NTf<sub>2</sub>]-1 and ILM-[NTf<sub>2</sub>]-3. At 80 ° C, the ring opening reaction of ILM-[NTf<sub>2</sub>]-3 requires 90 minutes to afford an epoxy conversion of about 90 % while ILM-[NTf<sub>2</sub>]-1 gives 76% after 2 hours. The lower conversion detected with ILM-[NTf<sub>2</sub>]-1 and ILM-[NTf<sub>2</sub>]-3 could be related to the nature of the terminal epoxides. By comparison with ILM-[NTf<sub>2</sub>]-2 which

incorporates an aromatic ring between imidazolium and epoxides, ILM-[NTf<sub>2</sub>]-1 and ILM-[NTf<sub>2</sub>]-3 have an epoxide directly linked to the imidazolium. In such cases, the short aliphatic chain could slow down the ring opening reaction. Finally, the presence of an ether group between the aromatic ring and the epoxyde is able to slightly modify the chemical kinetics too. Thus, the reaction between ILM-[NTf<sub>2</sub>]-2 and Jeffamine D230 during 35 minutes at 80 °C led to a full conversion of the epoxy functions opening some perspectives in the development of fast cure epoxy-amine systems. These results are in agreement with DSC curves highlighting the influence of the ILM-[NTf<sub>2</sub>] chemical nature on the epoxy prepolymer reactivity with the amine. For both ILM-1 and ILM-3, DSC thermograms showed the presence of two exothermic peaks indicating a two-step mechanism (See **Figure S22 and S24 in the ESI**). This phenomenon was already observed by Maka *et al* by using imidazolium ILs as hardeners of DGEBA prepolymer<sup>43,49</sup>. They have attributed this bimodal character to the adduct formation and chain addition polymerization<sup>42</sup>. Like conventional epoxy-amine networks, especially DGEBA cured with Jeffamine D230, a single exothermic peak was observed at 120 °C for ILM-2 (See **Figure 20** in the ESI) confirming its high reactivity as demonstrated by FTIR spectroscopy. In terms of enthalpies, the exothermicity generated during the polymerization between the ILM-[NTf<sub>2</sub>] and the amine is significantly lower compared to a conventional epoxy-amine reaction. Indeed, enthalpy values of 150 and 230 J.g<sup>-1</sup> were calculated for ILM-2, ILM-1 and ILM-3 compared to 550-600 J.g<sup>-1</sup> for DGEBA/D230 networks<sup>50-51</sup>.

In conclusion, epoxy-amine networks prepared from these three ILM-[NTf<sub>2</sub>] have shown similar reactivity's compared to DGEBA/D230 network combined with a full epoxy conversion.

**Determination of the relaxation temperature (T<sub>α</sub>) and approximation of the average molecular weight between crosslinks (M<sub>c</sub>) by dynamical mechanical analysis (DMA)**