International Journal of Advanced Chemistry, 6 (2) (2018) 167-189

International Journal of Advanced Chemistry

Website: www.sciencepubco.com/index.php/IJAC
doi: 10.14419/ijac.v6i2.12528
Research paper

Vibrational biospectroscopic study and chemical structure analysis of unsaturated polyamides nanoparticles as anti–cancer polymeric nanomedicines using synchrotron radiation

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Abstract

Firstly, unsaturated polyamides nanoparticles were hardened by continuous synchrotron radiation and then, the induced changes in its chemical structure were studied by Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectroscopy. It was shown that applying synchrotron radiation for hardening not only leads to reduction of hardening time but also creates cross link in polymer by breaking Carbon–Carbon double bond, without any considerable change in its chemical structure. In addition, an unsaturated polyamide nanoparticle as anti–cancer polymeric nanomedicines is hardened by synchrotron radiation. Its chemical structure before and after hardening is studied using Raman and Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectroscopy. The results show that Raman spectroscopy is considerably better than Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectroscopy in detecting the changes happened in chemical structure.

Keywords: *Unsaturated Polyamides Nanoparticles; Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) Spectroscopy; Carbon–Carbon Double Bond; Hardening–Cross Link; Cross Link; Raman Spectroscopy; Anti–Cancer Polymeric Nanomedicines; Synchrotron Radiation.*

1. Introduction

Polyamides nanoparticles as anti–cancer polymeric nanomedicines are one the most applicable polymers in the industry. Its hardening usually performs by adding a nanomaterial as hardener and heating them (Heidari and Brown 2018; Heidari 2016). In the current paper, Carbon–Carbon double bond presented in the structure is broken using continuous synchrotron radiation, without any damage to other presented bonds and unsaturated polyamides nanoparticles as anti–cancer polymeric nanomedicines is hardened by creating cross link (along with phase change from liquid to solid). In this regard, Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectroscopy was used to demonstrate breaking the bonds and no change in the structure of other bonds. It is worthwhile to note that in addition to above mentioned advantages, hardening with synchrotron radiation shortens the hardening time down to lower than 10 minutes. However, in traditional methods, which are mainly thermal methods, about 30 minutes is required for hardening and chemical structure changes during hardening process. The hardening process is molding not selective with small cross section. Unsaturated polyamides nanoparticles as anti–cancer polymeric nanomedicines is one of the most applicable known polymers which uses in liquid and hardened form for connections, molding, etc.

There are various spectroscopy methods to investigate chemical bonds of polyamides nanoparticles including Raman and Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectroscopy methods, which have their specific characteristics. Researches have been shown that some molecular symmetry cannot be detected by Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectroscopy while these can be detected by Raman spectroscopy. Polymers become harden due to creation of cross link in linear structure of polymer; these bonds are C–C type and cannot be detected by Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectroscopy while Raman spectroscopy is able to clearly detect these bonds. In the considered unsaturated polyamides nanoparticles structure, C=C bonds cannot be detected by Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectroscopy since this bond is symmetrical in linear structure of polymer. According to the reasons that are expressed in the following sections, the emerging region of this bond (1600–1700 cm^{-1}) is very important. Therefore, it is expected that these changes can be more clearly detected by Raman method. The aim of the current study is showing the efficiency of Raman method by comparing these two spectroscopy methods.

2. Chemical structure

In traditional methods, a hardener is always used for polymer hardening (its ratio for this polymer is 3 to 1) and it heats in 70°C for 30 minutes to harden the polymer. Hardening process is so that polymer changes to free radical by absorbing the required energy for breaking Carbon–Carbon double bond and then, make a bond with hardener (here, it is divinylbenzene) (Heidari 2016). The hardener can makes bond with two polymeric chains and it leads to creation of a cross link with the aid of hardener. Creation of cross link leads to change of polymer structure from linear to lattice structure and hence, it hardens the polymer. Chemical structure for unsaturated polyamides nanoparticles is shown in Figure (1). Moreover, its hardened form using (+)–Benzo(a)pyrene–7,8–dihydrodiol–9,10–epoxide is shown in Figure (2).

In the current paper, this double bond is broken by synchrotron radiation, without adding hardener, and cross link is created between linear chains of polymer. The utilized synchrotron radiation is continuous synchrocyclotron radiation with 20 (W) powers (Heidari 2016, 2017; Heidari and Brown 2017; Bastogne 2017; Vanić et al. 2013; Islan et al. 2017; Bawarski et al. 2008; Eaton et al. 2011; Hadinoto et al. 2014; Svenson et al. 2011; Sosnik et al. 2013; Filipović-Grčić et al. 2013; Yu et al. 2015; Moghimi et al. 2013; Eliasof et al. 2010; Domingo et al. 2012; Samadder et al. 2016; Yen et al. 2010; Azmi et al. 2016; L et al. 2015; Liu et al. 2012; Gabellieri et al. 2011; Frederickson 2016 et al; Namdari et al. 2017; Kiew et al. 2015; Moghimi et al. 2012; Gil et al. 2010; Rzigalinski et al. 2009; Fako et al. 2009; Sainz et al. 2015; Duncan et al. 2010; Zhou et al. 2014; Wibroe et al. 2016; Nguyen et al. 2016; Beija et al. 2012; Vaishali et al. 2017; Bawa 2009; Marianecci et al. 2016; Patil et al. 2017; Fonseca et al. 2014; Bedi et al. 2011; Canal et al. 2011; Hügel et al. 2014; Donaldson 2012; Bose et al. 2016; Hall et al. 2017; Storm 2012; du Toit et al. 2010; Kumar et al. 2016; Rajabi et al. 2016; Andersen et al. 2012; Kabanov et al. 2011; Nagy et al. 2015; Nickols-Richardson et al. 2007; Gaspar et al. 2009; Bourlinos et al. 2012; Svenson et al. 2012; Sitterberg et al. 2010; Telford 2005). Synchrotron radiation properties are listed in Table (1) (Heidari 2016, 2017; Heidari and Brown 2017; Bastogne 2017; Vanić et al. 2013; Islan et al. 2017; Bawarski et al. 2008; Eaton et al. 2011; Hadinoto et al. 2014; Svenson et al. 2011; Sosnik et al. 2013; Filipović-Grčić et al. 2013; Yu et al. 2015; Moghimi et al. 2013; Eliasof et al. 2010; Domingo et al. 2012; Samadder et al. 2016; Yen et al. 2010; Azmi et al. 2016; L et al. 2015; Liu et al. 2012; Gabellieri et al. 2011; Frederickson 2016 et al; Namdari et al. 2017; Kiew et al. 2015; Moghimi et al. 2012; Gil et al. 2010; Rzigalinski et al. 2009; Fako et al. 2009; Sainz et al. 2015; Duncan et al. 2010; Zhou et al. 2014; Wibroe et al. 2016; Nguyen et al. 2016; Beija et al. 2012; Vaishali et al. 2017; Bawa 2009; Marianecci et al. 2016; Patil et al. 2017; Fonseca et al. 2014; Bedi et al. 2011; Canal et al. 2011; Hügel et al. 2014; Donaldson 2012; Bose et al. 2016; Hall et al. 2017; Storm 2012; du Toit et al. 2010; Kumar et al. 2016; Rajabi et al. 2016; Andersen et al. 2012; Kabanov et al. 2011; Nagy et al. 2015; Nickols-Richardson et al. 2007; Gaspar et al. 2009; Bourlinos et al. 2012; Svenson et al. 2012; Sitterberg et al. 2010; Telford 2005).

As mentioned before, the polymer used in the current study is unsaturated polyamides nanoparticles with chemical structure shown in Figure (1). These polyamides nanoparticles can be hardened by synchrotron radiation for 3 to 6 minutes (Alibolandi et al. 2015; Bridoux et al. 2009; Stuurman et al. 2010; Kondo 2010; Jindal et al. 2017; Rapoport 2007; Fernández 2011; Pippa et al. 2013; Verreault et al. 2012; Hassanzadeh et al. 2017; Sivanesan et al. 2017; Phillips et al. 2010; Varan et al. 2017; Moghimi et al. 2014; Soria et al. 2010; McMurray et al. 2010; Sans-Serramitjana et al. 2016; Rigo et al. 2017; Alibolandi et al. 2017; Bridoux et al. 2010; Tutaj et al. 2016; Kuppusamy et al. 2013; Tomalia 2006; Menjoge et al. 2010; Vega-Villa et al. 2008; Gaur et al. 2014; Tietze et al. 2015; Schwengber et al. 2015; Adhikari et al. 2017; Szebeni et al. 2015; Chen et al. 2011; Requejo-Aguilar et al. 2017; Golyshkin et al. 2016; Szulc et al. 2016; Haddad et al. 2008; Mignani et al. 2013; Eaton et al. 2015; Lollo et al. 2015; Thompson et al. 2012; Muntimadugu et al. 2017; Foldvari et al. 2008; Riley et al. 2012; Fernandes et al. 2015; Mehra et al. 2016; Mignani et al. 2016; Naderkhani et al. 2014; Newton 2013; Aoki et al. 2015; Ita 2014; Liu et al. 2008; Mallapragada et al. 2015; Peres et al. 2017; Ferreira et al. 2013; Salerno et al. 2015; Tyler et al. 2016; Iannazzo et al. 2015; Jemec et al. 2012; Chen et al. 2012; Lütscher et al. 2012; Park et al. 2013; Huang et al. 2011; Depan et al. 2011; Guo et al. 2014; Duncan 2011; Sidik et al. 2016; Yuan et al. 2010; He et al. 2014; An et al. 2013; Meenach et al. 2013; England et al. 2012; Pippa et al. 2013; Boisseau et al. 2011; Petrichenko et al. 2015; Rodríguez-Gascón et al. 2015; Frima et al. 2012; Yallapu et al. 2015; Duan et al. 2012; Perez et al. 2015; Costantino et al. 2012; Wei et al. 2006; Murday et al. 2009; Dixit et al. 2015; Nair et al. 2010; Bawa et al. 2005; Farkhani et al. 2014; Lal et al. 2010; Hacklin et al. 2009; Gabizon et al. 2016; Zhang et al. 2013; Vanić et al. 2014; Ellis-Behnke 2007; Srivalli et al. 2016; Collnot et al. 2012; Rychak et al. 2006; Watala et al. 2016; Palombo et al. 2009; Kuzmov et al. 2015; Diebold et al. 2010; Bal et al. 2011; Bharali et al. 2010; Ray et al. 2010; Mishra et al. 2010; Torchilin 2009; Cupaioli et al. 2014; Sosnik et al. 2014; Guan et al. 2013; Toit et al. 2013; Zhang et al. 2012; Muthaiyan et al. 2011; Duncan 2009; Palao-Suay et al. 2016; Morrow et al. 2007; Punetha et al. 2017; Manickam 2017; Osorio et al. 2015; Karami et al. 2016; Park et al. 2013; Heidari 2017, 2018; Gobato et al. 2018; Gobato, Heidari 2018; Heidari, Gobato 2018). As we know, polyamides nanoparticles are hardened due to creation of C–C cross links in the structure of polyamides nanoparticles. These bonds are resulted from breaking Carbon double bond (C=C) and can connect two linear structure of polyamides nanoparticles to each other (Alibolandi et al. 2015; Bridoux et al. 2009; Stuurman et al. 2010; Kondo 2010; Jindal et al. 2017; Rapoport 2007; Fernández 2011; Pippa et al. 2013; Verreault et al. 2012; Hassanzadeh et al. 2017; Sivanesan et al. 2017; Phillips et al. 2010; Varan et al. 2017; Moghimi et al. 2014; Soria et al. 2010; McMurray et al. 2010; Sans-Serramitjana et al. 2016; Rigo et al. 2017; Alibolandi et al. 2017; Bridoux et al. 2010; Tutaj et al. 2016; Kuppusamy et al. 2013; Tomalia 2006; Menjoge et al. 2010; Vega-Villa et al. 2008; Gaur et al. 2014; Tietze et al. 2015; Schwengber et al. 2015; Adhikari et al. 2017; Szebeni et al. 2015; Chen et al. 2011; Requejo-Aguilar et al. 2017; Golyshkin et al. 2016; Szulc et al. 2016; Haddad et al. 2008; Mignani et al. 2013; Eaton et al. 2015; Lollo et al. 2015; Thompson et al. 2012; Muntimadugu et al. 2017; Foldvari et al. 2008; Riley et al. 2012; Fernandes et al. 2015; Mehra et al. 2016; Mignani et al. 2016; Naderkhani et al. 2014; Newton 2013; Aoki et al. 2015; Ita 2014; Liu et al. 2008; Mallapragada et al. 2015; Peres et al. 2017; Ferreira et al. 2013; Salerno et al. 2015; Tyler et al. 2016; Iannazzo et al. 2015; Jemec et al. 2012; Chen et al. 2012; Lütscher et al. 2012; Park et al. 2013; Huang et al. 2011; Depan et al. 2011; Guo et al. 2014; Duncan 2011; Sidik et al. 2016; Yuan et al. 2010; He et al. 2014; An et al. 2013; Meenach et al. 2013; England et al. 2012; Pippa et al. 2013; Boisseau et al. 2011; Petrichenko et al. 2015; Rodríguez-Gascón et al. 2015; Frima et al. 2012; Yallapu et al. 2015; Duan et al. 2012; Perez et al. 2015; Costantino et al. 2012; Wei et al. 2006; Murday et al. 2009; Dixit et al. 2015; Nair et al. 2010; Bawa et al. 2005; Farkhani et al. 2014; Lal et al. 2010; Hacklin et al. 2009; Gabizon et al. 2016; Zhang et al. 2013; Vanić et al. 2014; Ellis-Behnke 2007; Srivalli et al. 2016; Collnot et al. 2012; Rychak et al. 2006; Watala et al. 2016; Palombo et al. 2009; Kuzmov et al. 2015; Diebold et al. 2010; Bal et al. 2011; Bharali et al. 2010; Ray et al. 2010; Mishra et al. 2010; Torchilin 2009; Cupaioli et al. 2014; Sosnik et al. 2014; Guan et al. 2013; Toit et al. 2013; Zhang et al. 2012; Muthaiyan et al. 2011; Duncan 2009; Palao-Suay et al. 2016; Morrow et al. 2007; Punetha et al. 2017; Manickam 2017; Osorio et al. 2015; Karami et al. 2016; Park et al. 2013; Heidari 2017, 2018; Gobato et al. 2018; Gobato, Heidari 2018; Heidari, Gobato 2018). In chemical methods, titration method is used to demonstrate such bond and hardening so that both hardening and breaking of C=C bond can be determined. The aim of the current study is observing and calculating the breaking and creation of cross link in a hardened polyamides nanoparticles (Figure (3)) using synchrotron radiation and comparing it with the results obtained from Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectroscopy.

3. Experimental work

To do this work, the considered polyamides nanoparticles as anti–cancer polymeric nanomedicines was subjected to synchrotron radiation for 3 and 6 minutes so that it was completely hardened (Alibolandi et al. 2015; Bridoux et al. 2009; Stuurman et al. 2010;

Kondo 2010; Jindal et al. 2017; Rapoport 2007; Fernández 2011; Pippa et al. 2013; Verreault et al. 2012; Hassanzadeh et al. 2017; Sivanesan et al. 2017; Phillips et al. 2010; Varan et al. 2017; Moghimi et al. 2014; Soria et al. 2010; McMurray et al. 2010; Sans-Serramitjana et al. 2016; Rigo et al. 2017; Alibolandi et al. 2017; Bridoux et al. 2010; Tutaj et al. 2016; Kuppusamy et al. 2013; Tomalia 2006; Menjoge et al. 2010; Vega-Villa et al. 2008; Gaur et al. 2014; Tietze et al. 2015; Schwengber et al. 2015; Adhikari et al. 2017; Szebeni et al. 2015; Chen et al. 2011; Requejo-Aguilar et al. 2017; Golyshkin et al. 2016; Szulc et al. 2016; Haddad et al. 2008; Mignani et al. 2013; Eaton et al. 2015; Lollo et al. 2015; Thompson et al. 2012; Muntimadugu et al. 2017; Foldvari et al. 2008; Riley et al. 2012; Fernandes et al. 2015; Mehra et al. 2016; Mignani et al. 2016; Naderkhani et al. 2014; Newton 2013; Aoki et al. 2015; Ita 2014; Liu et al. 2008; Mallapragada et al. 2015; Peres et al. 2017; Ferreira et al. 2013; Salerno et al. 2015; Tyler et al. 2016; Iannazzo et al. 2015; Jemec et al. 2012; Chen et al. 2012; Lütscher et al. 2012; Park et al. 2013; Huang et al. 2011; Depan et al. 2011; Guo et al. 2014; Duncan 2011; Sidik et al. 2016; Yuan et al. 2010; He et al. 2014; An et al. 2013; Meenach et al. 2013; England et al. 2012; Pippa et al. 2013; Boisseau et al. 2011; Petrichenko et al. 2015; Rodríguez-Gascón et al. 2015; Frima et al. 2012; Yallapu et al. 2015; Duan et al. 2012; Perez et al. 2015; Costantino et al. 2012; Wei et al. 2006; Murday et al. 2009; Dixit et al. 2015; Nair et al. 2010; Bawa et al. 2005; Farkhani et al. 2014; Lal et al. 2010; Hacklin et al. 2009; Gabizon et al. 2016; Zhang et al. 2013; Vanić et al. 2014; Ellis-Behnke 2007; Srivalli et al. 2016; Collnot et al. 2012; Rychak et al. 2006; Watala et al. 2016; Palombo et al. 2009; Kuzmov et al. 2015; Diebold et al. 2010; Bal et al. 2011; Bharali et al. 2010; Ray et al. 2010; Mishra et al. 2010; Torchilin 2009; Cupaioli et al. 2014; Sosnik et al. 2014; Guan et al. 2013; Toit et al. 2013; Zhang et al. 2012; Muthaiyan et al. 2011; Duncan 2009; Palao-Suay et al. 2016; Morrow et al. 2007; Punetha et al. 2017; Manickam 2017; Osorio et al. 2015; Karami et al. 2016; Park et al. 2013; Heidari 2017, 2018; Gobato et al. 2018; Gobato, Heidari 2018; Heidari, Gobato 2018).

Now, Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectroscopy was used to investigate the changes happened in the chemical structure of polyamides nanoparticles. To qualitatively investigate the changes happened during radiation, a specific amount of polymer was placed on KBr pill and its spectrum was recorded. Then, pill was subjected to synchrotron radiation for 3 minutes and then, spectrum was again recorded and this process was repeated for another 3 minutes. Therefore, three graphs were available which have similar mass ratio, as shown in Figure (4). In this figure, Carbon–Carbon double bond (C=C) at the range of 1600–1700 (cm^{-1}) is shown as two peaks. The peak at 1630 (cm^{-1}) is related to double bond C=C bond and the peak at 1644 (cm^{-1}) is related to conjugated C=C bond. It is expected that double bond bond eliminates during radiation. In addition, the peak at 1725.5 (cm^{-1}) which is related to Carbon–Oxygen double bond (C=O) is expected to emerge at 1715 (cm^{-1}). However, due to the presence of resonance, which is happened because of adjoining a Carbonyl group (C=C) with an Ester group (C=O) (similar to Figure (5)), it emerges in this region. It is expected that the shift induced by resonance reduces and the peak related to C=O bond shifts back to its original position as C=C bond eliminates (Table (2)) (Alibolandi et al. 2015; Bridoux et al. 2009; Stuurman et al. 2010; Kondo 2010; Jindal et al. 2017; Rapoport 2007; Fernández 2011; Pippa et al. 2013; Verreault et al. 2012; Hassanzadeh et al. 2017; Sivanesan et al. 2017; Phillips et al. 2010; Varan et al. 2017; Moghimi et al. 2014; Soria et al. 2010; McMurray et al. 2010; Sans-Serramitjana et al. 2016; Rigo et al. 2017; Alibolandi et al. 2017; Bridoux et al. 2010; Tutaj et al. 2016; Kuppusamy et al. 2013; Tomalia 2006; Menjoge et al. 2010; Vega-Villa et al. 2008; Gaur et al. 2014; Tietze et al. 2015; Schwengber et al. 2015; Adhikari et al. 2017; Szebeni et al. 2015; Chen et al. 2011; Requejo-Aguilar et al. 2017; Golyshkin et al. 2016; Szulc et al. 2016; Haddad et al. 2008; Mignani et al. 2013; Eaton et al. 2015; Lollo et al. 2015; Thompson et al. 2012; Muntimadugu et al. 2017; Foldvari et al. 2008; Riley et al. 2012; Fernandes et al. 2015; Mehra et al. 2016; Mignani et al. 2016; Naderkhani et al. 2014; Newton 2013; Aoki et al. 2015; Ita 2014; Liu et al. 2008; Mallapragada et al. 2015; Peres et al. 2017; Ferreira et al. 2013; Salerno et al. 2015; Tyler et al. 2016; Iannazzo et al. 2015; Jemec et al. 2012; Chen et al. 2012; Lütscher et al. 2012; Park et al. 2013; Huang et al. 2011; Depan et al. 2011; Guo et al. 2014; Duncan 2011; Sidik et al. 2016; Yuan et al. 2010; He et al. 2014; An et al. 2013; Meenach et al. 2013; England et al. 2012; Pippa et al. 2013; Boisseau et al. 2011; Petrichenko et al. 2015; Rodríguez-Gascón et al. 2015; Frima et al. 2012; Yallapu et al. 2015; Duan et al. 2012; Perez et al. 2015; Costantino et al. 2012; Wei et al. 2006; Murday et al. 2009; Dixit et al. 2015; Nair et al. 2010; Bawa et al. 2005; Farkhani et al. 2014; Lal et al. 2010; Hacklin et al. 2009; Gabizon et al. 2016; Zhang et al. 2013; Vanić et al. 2014; Ellis-Behnke 2007; Srivalli et al. 2016; Collnot et al. 2012; Rychak et al. 2006; Watala et al. 2016; Palombo et al. 2009; Kuzmov et al. 2015; Diebold et al. 2010; Bal et al. 2011; Bharali et al. 2010; Ray et al. 2010; Mishra et al. 2010; Torchilin 2009; Cupaioli et al. 2014; Sosnik et al. 2014; Guan et al. 2013; Toit et al. 2013; Zhang et al. 2012; Muthaiyan et al. 2011; Duncan 2009; Palao-Suay et al. 2016; Morrow et al. 2007; Punetha et al. 2017; Manickam 2017; Osorio et al. 2015; Karami et al. 2016; Park et al. 2013; Heidari 2017, 2018; Gobato et al. 2018; Gobato, Heidari 2018; Heidari, Gobato 2018).

In Figure (6), energy range of 1540–1680 (cm^{-1}) is magnified so that the changes can be more clearly seen (Figure (8)).

As it is expected, double bond is eliminated and conjugated bond is considerably reduced. To calculate the remained amount of double bond, a reference peak (a bond that is not changed) is used that is related to Carbon–Oxygen double bond (Ester function C=O) and is shown as a large peak at 1725 (cm^{-1}). In this regard, the area below the curves of C=O and C=C are calculated in each step, by Origin latest version 2018 software, and the obtained results are compared with each other.

If the obtained ratio from the curve of liquid (black curve) is considered as reference value, it can be said that after 3 and 6 minutes of radiation, about 49% and 35%, respectively, of double bonds are remained. Therefore, it seems that double bond is broken and cross link is created. Changes in color and not solving in initial solvent (acetone) can be considered as the signs of this issue. To show that the considered method is applicable for all unsaturated polyamides nanoparticles as anti–cancer polymeric nanomedicines, this process was repeated for general polyamides nanoparticles as anti–cancer polymeric nanomedicines and again, it was radiated for 6 minutes with similar mass ratio.

Again, C=O bond was considered as reference and previous works were repeated to obtain the remained amount of C=C bonds. In this nanomaterial, the remained amount of C=C bond after 6 minutes of radiation is about 48%.

For quantitative evaluation of the changes happened in the presented peaks in Raman and Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectra, it is enough to harden a small amount of nanomaterial and obtain its spectrum before and after hardening.

For Raman spectroscopy, a given amount of the nanomaterial is selected and its spectrum is obtained. Then, an equal mass of nanomaterial is hardened and its spectrum is obtained again (Figure 9). For Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectrum, a given amount of nanomaterial is placed over KBr pill and then, its spectrum is obtained. Then, the pill is subjected to synchrotron radiation for 6 minutes and then its spectrum is obtained to create Figure (10) (Alibolandi et al. 2015; Bridoux et al. 2009; Stuurman et al. 2010; Kondo 2010; Jindal et al. 2017; Rapoport 2007; Fernández 2011; Pippa et al. 2013; Verreault et al. 2012; Hassanzadeh et al. 2017; Sivanesan et al. 2017; Phillips et al. 2010; Varan et al. 2017; Moghimi et al. 2014; Soria et al. 2010; McMurray et al. 2010; Sans-Serramitjana et al. 2016; Rigo et al. 2017; Alibolandi et al. 2017; Bridoux et al. 2010; Tutaj et al. 2016; Kuppusamy et al. 2013; Tomalia 2006; Menjoge et al. 2010; Vega-Villa et al. 2008; Gaur et al. 2014; Tietze et al.

2015; Schwengber et al. 2015; Adhikari et al. 2017; Szebeni et al. 2015; Chen et al. 2011; Requejo-Aguilar et al. 2017; Golyshkin et al. 2016; Szulc et al. 2016; Haddad et al. 2008; Mignani et al. 2013; Eaton et al. 2015; Lollo et al. 2015; Thompson et al. 2012; Muntimadugu et al. 2017; Foldvari et al. 2008; Riley et al. 2012; Fernandes et al. 2015; Mehra et al. 2016; Mignani et al. 2016; Naderkhani et al. 2014; Newton 2013; Aoki et al. 2015; Ita 2014; Liu et al. 2008; Mallapragada et al. 2015; Peres et al. 2017; Ferreira et al. 2013; Salerno et al. 2015; Tyler et al. 2016; Iannazzo et al. 2015; Jemec et al. 2012; Chen et al. 2012; Lütscher et al. 2012; Park et al. 2013; Huang et al. 2011; Depan et al. 2011; Guo et al. 2014; Duncan 2011; Sidik et al. 2016; Yuan et al. 2010; He et al. 2014; An et al. 2013; Meenach et al. 2013; England et al. 2012; Pippa et al. 2013; Boisseau et al. 2011; Petrichenko et al. 2015; Rodríguez-Gascón et al. 2015; Frima et al. 2012; Yallapu et al. 2015; Duan et al. 2012; Perez et al. 2015; Costantino et al. 2012; Wei et al. 2006; Murday et al. 2009; Dixit et al. 2015; Nair et al. 2010; Bawa et al. 2005; Farkhani et al. 2014; Lal et al. 2010; Hacklin et al. 2009; Gabizon et al. 2016; Zhang et al. 2013; Vanić et al. 2014; Ellis-Behnke 2007; Srivalli et al. 2016; Collnot et al. 2012; Rychak et al. 2006; Watala et al. 2016; Palombo et al. 2009; Kuzmov et al. 2015; Diebold et al. 2010; Bal et al. 2011; Bharali et al. 2010; Ray et al. 2010; Mishra et al. 2010; Torchilin 2009; Cupaioli et al. 2014; Sosnik et al. 2014; Guan et al. 2013; Toit et al. 2013; Zhang et al. 2012; Muthaiyan et al. 2011; Duncan 2009; Palao-Suay et al. 2016; Morrow et al. 2007; Punetha et al. 2017; Manickam 2017; Osorio et al. 2015; Karami et al. 2016; Park et al. 2013; Heidari 2017, 2018; Gobato et al. 2018; Gobato, Heidari 2018; Heidari, Gobato 2018).

To investigate the hardening process, the region in which C=O, C–O and C=C bonds can be observed is considered (900–1800 cm^{-1}). Using Origin software and experimental data, this range can be depicted as shown in Figures (10) and (11).

As is expected, C=C bond is considerably synchrotron radiation than C=O bond in Raman spectrum. Further, C–C bond that is not shown in Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectrum can be clearly observed in this spectrum in the range of 900–1300 (cm^{-1}) (Figure (7)). As the main reason for creation of cross link is elimination of this bond and its converting to C–C cross link, variations at this peak (1624 cm^{-1}) and creation of new bonds in the range related to C–C bonds is very important. Firstly, the changes observed between liquid and hardened polymer in Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectrum are considered. As can be seen in Figure (10), C=C bond emerges at 1644 (cm^{-1}). After hardening, the heights of peaks are considerably reduced (due to clouding of nanomaterial). As the height of peak is strongly depend on the concentration of nanomaterial, it cannot be the only criterion for breaking the bonds. To demonstrate the reduction in number of bonds, the ratio of area below the related curve to C=C bond and the related curve to a bond that is not changed during hardening before and after hardening process are calculated (Alibolandi et al. 2015; Bridoux et al. 2009; Stuurman et al. 2010; Kondo 2010; Jindal et al. 2017; Rapoport 2007; Fernández 2011; Pippa et al. 2013; Verreault et al. 2012; Hassanzadeh et al. 2017; Sivanesan et al. 2017; Phillips et al. 2010; Varan et al. 2017; Moghimi et al. 2014; Soria et al. 2010; McMurray et al. 2010; Sans-Serramitjana et al. 2016; Rigo et al. 2017; Alibolandi et al. 2017; Bridoux et al. 2010; Tutaj et al. 2016; Kuppusamy et al. 2013; Tomalia 2006; Menjoge et al. 2010; Vega-Villa et al. 2008; Gaur et al. 2014; Tietze et al. 2015; Schwengber et al. 2015; Adhikari et al. 2017; Szebeni et al. 2015; Chen et al. 2011; Requejo-Aguilar et al. 2017; Golyshkin et al. 2016; Szulc et al. 2016; Haddad et al. 2008; Mignani et al. 2013; Eaton et al. 2015; Lollo et al. 2015; Thompson et al. 2012; Muntimadugu et al. 2017; Foldvari et al. 2008; Riley et al. 2012; Fernandes et al. 2015; Mehra et al. 2016; Mignani et al. 2016; Naderkhani et al. 2014; Newton 2013; Aoki et al. 2015; Ita 2014; Liu et al. 2008; Mallapragada et al. 2015; Peres et al. 2017; Ferreira et al. 2013; Salerno et al. 2015; Tyler et al. 2016; Iannazzo et al. 2015; Jemec et al. 2012; Chen et al. 2012; Lütscher et al. 2012; Park et al. 2013; Huang et al. 2011; Depan et al. 2011; Guo et al. 2014; Duncan 2011; Sidik et al. 2016; Yuan et al. 2010; He et al. 2014; An et al. 2013; Meenach et al. 2013; England et al. 2012; Pippa et al. 2013; Boisseau et al. 2011; Petrichenko et al. 2015; Rodríguez-Gascón et al. 2015; Frima et al. 2012; Yallapu et al. 2015; Duan et al. 2012; Perez et al. 2015; Costantino et al. 2012; Wei et al. 2006; Murday et al. 2009; Dixit et al. 2015; Nair et al. 2010; Bawa et al. 2005; Farkhani et al. 2014; Lal et al. 2010; Hacklin et al. 2009; Gabizon et al. 2016; Zhang et al. 2013; Vanić et al. 2014; Ellis-Behnke 2007; Srivalli et al. 2016; Collnot et al. 2012; Rychak et al. 2006; Watala et al. 2016; Palombo et al. 2009; Kuzmov et al. 2015; Diebold et al. 2010; Bal et al. 2011; Bharali et al. 2010; Ray et al. 2010; Mishra et al. 2010; Torchilin 2009; Cupaioli et al. 2014; Sosnik et al. 2014; Guan et al. 2013; Toit et al. 2013; Zhang et al. 2012; Muthaiyan et al. 2011; Duncan 2009; Palao-Suay et al. 2016; Morrow et al. 2007; Punetha et al. 2017; Manickam 2017; Osorio et al. 2015; Karami et al. 2016; Park et al. 2013; Heidari 2017, 2018; Gobato et al. 2018; Gobato, Heidari 2018; Heidari, Gobato 2018).

As can be observed, decrease in this ratio shows that Carbon double bond (C=C) is broken. In fact, the remaining amount of Carbon double bond is 33.3% after 6 minutes of synchrotron radiation. In this curve, it is not possible to directly observe creation of cross links and it is only possible to indirectly guess the creation of some bonds in C–O structure. Further, low height of peak related to C=C makes it impossible to clearly observe the changes induced by breaking the bond. However, the shift in peaks towards longer wavelengths is another reason for breaking Carbon double bonds and creation of cross links (in fact, the existence of resonance in chemical structure that is made from adjoining C=O and C=C bonds leads to shifting the peak of C=O from 1715 (cm^{-1}) to 1725 (cm^{-1}). Therefore, by elimination of double bond it is expected that this bond shifts back to its original position) (Figure (11) and Table (2)). However, there are various interesting points in Raman spectrum. The ratios of peak reduction are:

The remaining C=C bond derived from these ratios is 12.5%. Such large difference between the obtained ratios from Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectrum (33.3%) and Raman spectrum (12.5%) indicates that the changes happened in this bond is considerably better observed in Raman spectrum than Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) one. To better understand the potentials of Raman, see Figure (10). As can be seen, in the range of 1000–1300 (cm^{-1}) related to C–C bond, it is possible to clearly observe cross links with increase in the number of peaks that are overlapped. In addition, converting the peak at 1471 (cm^{-1}) that is related to CH_2 and CH_3 structures to two peaks and addition of peak at 1423.43 (cm^{-1}) (related to CH_3 structure) because of the presence of CH_3 at the end of the chain, indicate the shortening of polymeric chain. Decreasing the peak at 99.4 (cm^{-1}) (C–O–C structure) confirms that linear structure of polymer is broken in a specific position and this is the reason for shortening the chain length and amorphous chemical structure.

Regarding the fact that this hardening method reduces the hardening time down to 7 minutes and considering this point that the chemical structure of polyamides nanoparticles are not mainly changed, against traditional methods (which use hardener nanomaterial), except that in the region of creation of cross links, it is proposed that this method can be used for capsulation and lithography. In addition, the following points can be derived from the differences between Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) and Raman spectra.

(I) Raman spectroscopy is the best method for detecting the bonds in non–polarized nanomolecules, especially the nanomolecules that are not detectable in traditional methods due to the symmetry of chemical structure. Opposite to Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectroscopy which shows rotational and vibration modes of polarized bonds such as C–H and C–O, Raman spectrum is able to show non–polarized structures such as C–C and C–O–C.

(II) It is better that both Raman and Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectra obtain from the sample. If both Raman and Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectra obtain, it can be seen that these two spectra are supplement of each other. Finally, it can be said that Raman spectroscopy is better than Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectroscopy since C=C and C–C bonds cannot be clearly observed in Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectroscopy. However, the synchrotron radiation amount in Raman spectroscopy should not be so high that leads to change in chemical structure.

4. Conclusion

As can be seen in Figure (1), except that C=C bonds at 1630 and 1644 (cm^{-1}), remained bonds have not change and it can be clearly seen from constant area ratio related to reference peak (C=O), before and after radiation. Except that bonds which are indirectly related to C=C bond, such as vinyl Hydrogen, that its considerable reduction is the sign of major changes in C=C, cross–links are not observed in spectroscopy. However, the changes happened in C–O bonds in the range of 1000–1300 (cm^{-1}) show some changes in linear structure of polyamides nanoparticles. It is expected that the mentioned bonds (C=C and C–C) will be more clearly emerged in Raman spectroscopy since the symmetry in these structures (Figure (2)) leads to inefficiency of Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR) spectroscopy. Meanwhile, decreasing the peak height in each step is due to clouding of polymer and increasing the reflection from polymer surface. The bond energy of C=C bond is about 611 (kJ). As 20% of synchrotron radiation wavelength (10.6 μm) is absorbed by nanomaterial and synchrotron radiation is radiated with enough time, the energy required for breaking the bond is provided for the nanomaterial. Creation of free radicals and high energy of bond for recombination of C=C bond lead to bonds with lower energy and as free radicals are happened over Carbon double bond, this bond is C–C cross–link.

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Figures' Captions:

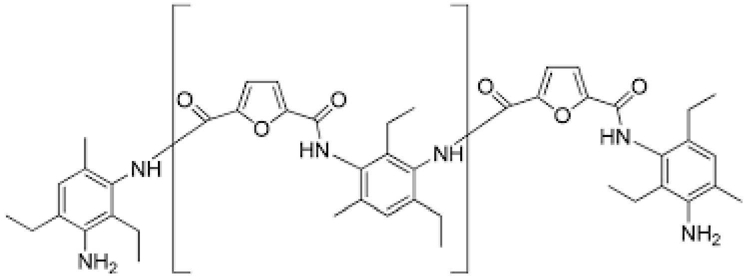


Fig. 1: Unsaturated polyamides nanoparticles chemical structure.

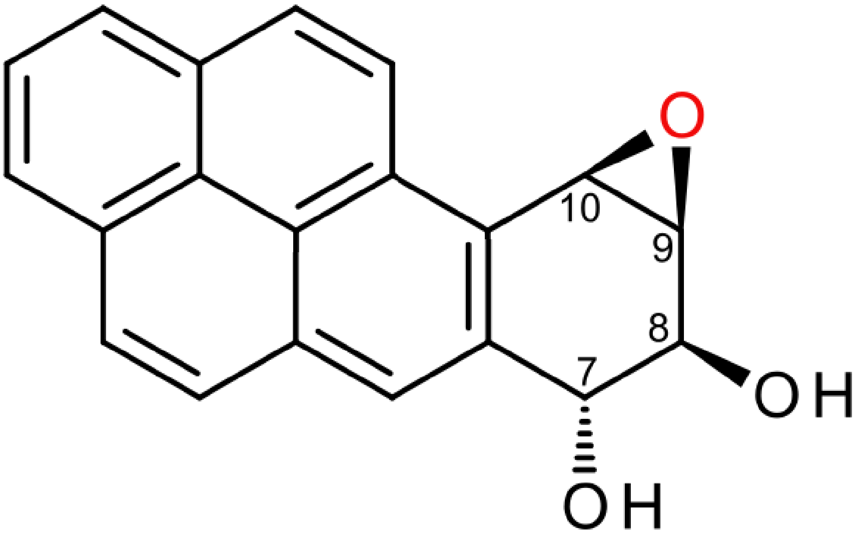
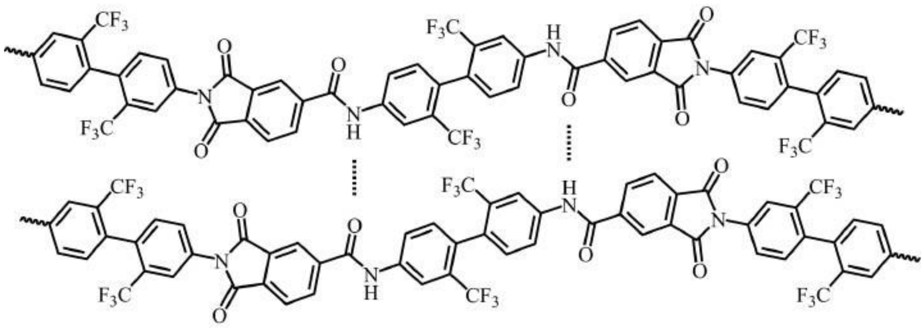
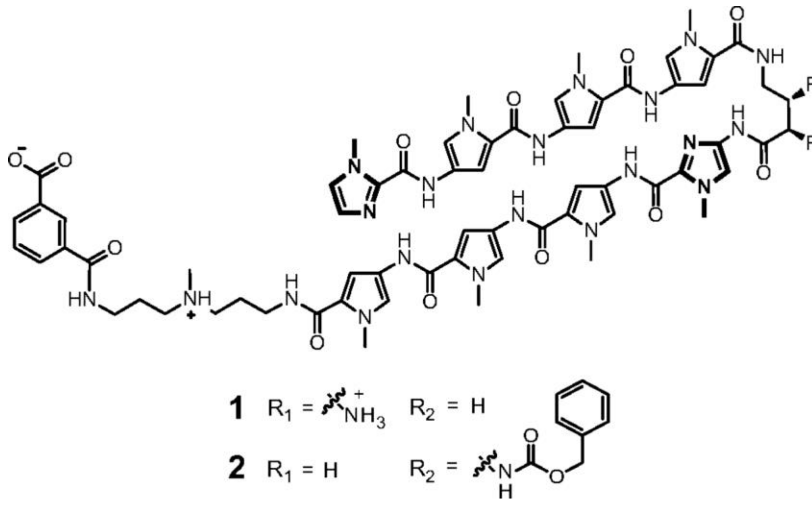


Fig. 2: Creation of cross link by hardener ((+)–Benzo(a)pyrene–7,8–dihydrodiol–9,10–epoxide).



(a)



(b)

Fig. 3: (a) Liquid and (b) hardened polyamides nanoparticles.

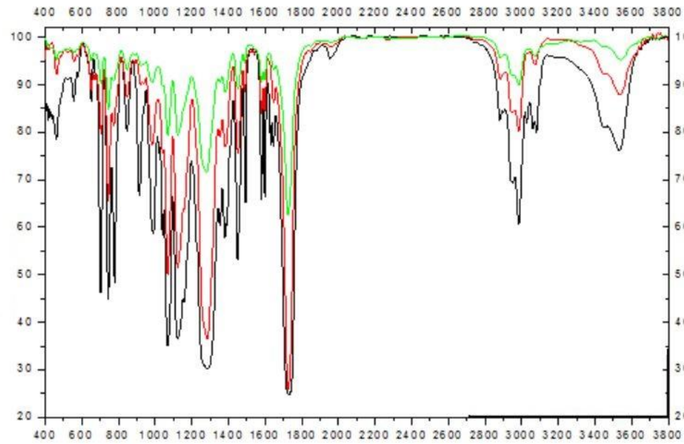


Fig. 4: ATR–FTIR spectra for three samples. Black is the sample without synchrotron radiation, red with 3 minutes and green with 6 minutes of synchrotron radiation. It should be noted that and x–axis shows wavenumber (cm^{-1}) and y–axis shows intensity, respectively.

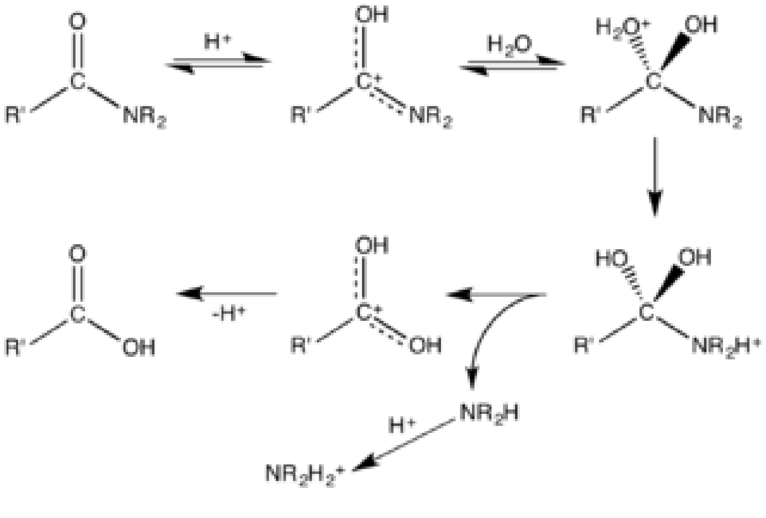


Fig. 5: Resonance created in chemical structure.

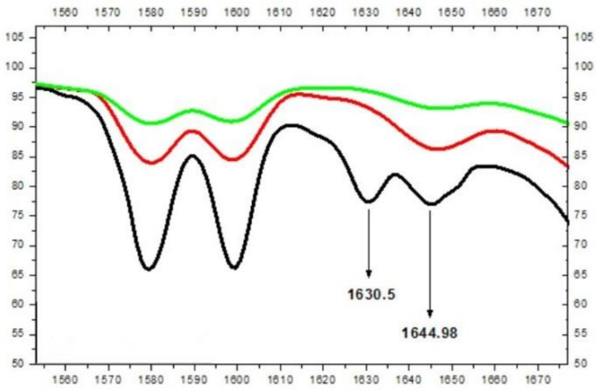


Fig. 6: Magnified range of 1540–1680 (cm^{-1}) to show the changes happened in the structure of double bond. It should be noted that and x–axis shows wavenumber (cm^{-1}) and y–axis shows intensity, respectively.

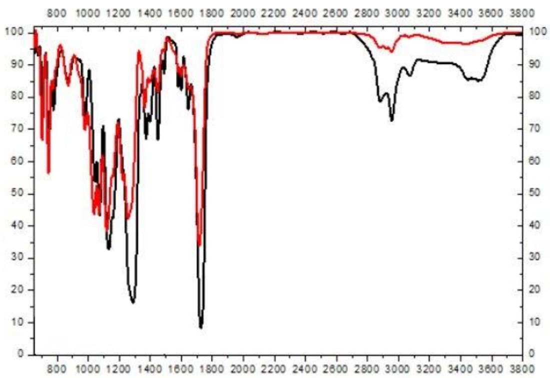


Fig. 7: ATR–FTIR spectrum for general unsaturated polyamides nanoparticles. C=C bond is seen as a peak at 1644 (cm^{-1}). It should be noted that and x–axis shows wavenumber (cm^{-1}) and y–axis shows intensity, respectively.

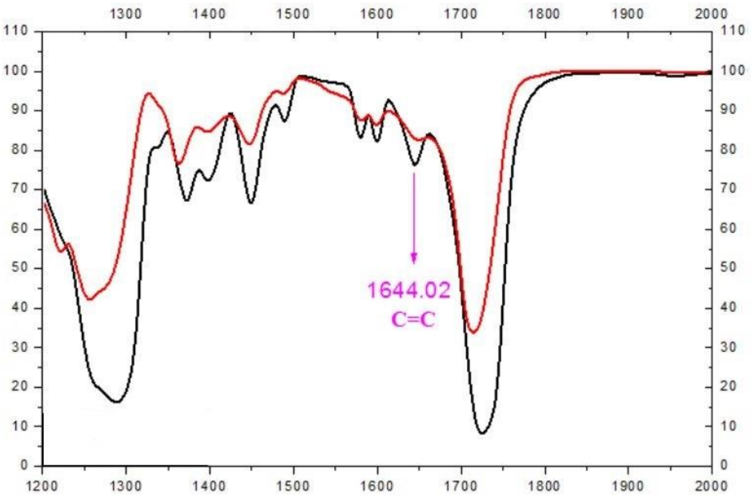


Fig. 8: Magnified range of 1200–2000 (cm^{-1}) to show the reduction and shift of peak at 1644 and 1725 (cm^{-1}). It should be noted that and x–axis shows wavenumber (cm^{-1}) and y–axis shows intensity, respectively.

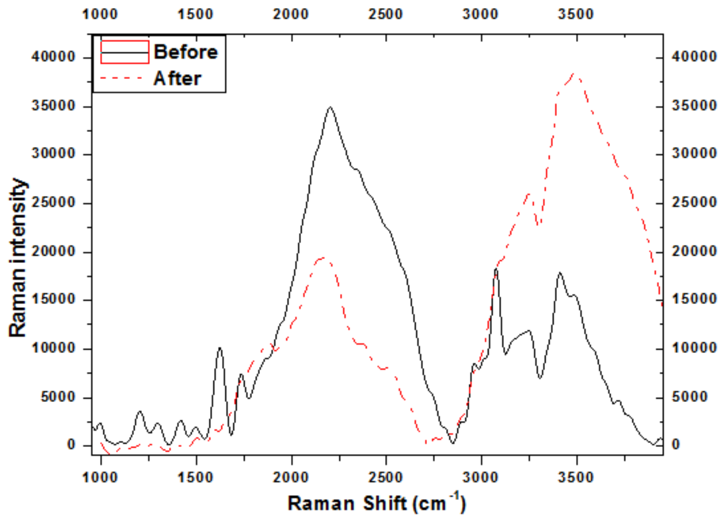


Fig. 9: Raman spectrum for polymer, dotted line is for after hardening and solid line is for before hardening. It should be noted that and x–axis shows wavenumber (cm^{-1}) and y–axis shows intensity, respectively.

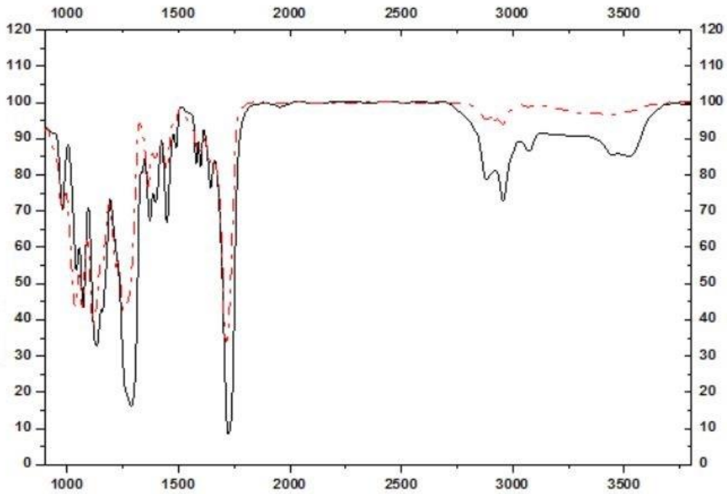


Fig. 10: ATR–FTIR spectrum, solid lines are related to liquid polymer and dotted lines are related to hardened polymer with synchrotron radiation. It should be noted that and x–axis shows wavenumber (cm^{-1}) and y–axis shows intensity, respectively.

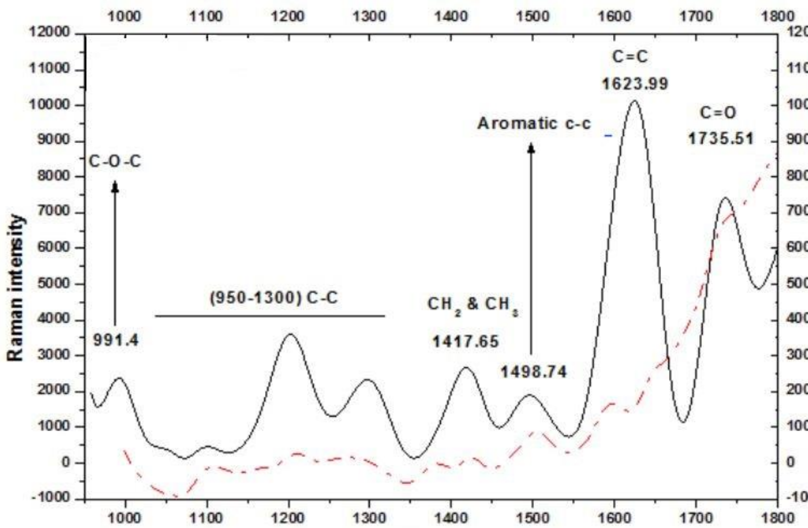


Fig. 11: Raman spectra before and after hardening are superimposed and peaks at 1623.99 and 1735.51 (cm^{-1}) are related to C=C and C=O, respectively. It should be noted that and x–axis shows wavenumber (cm^{-1}) and y–axis shows intensity, respectively.

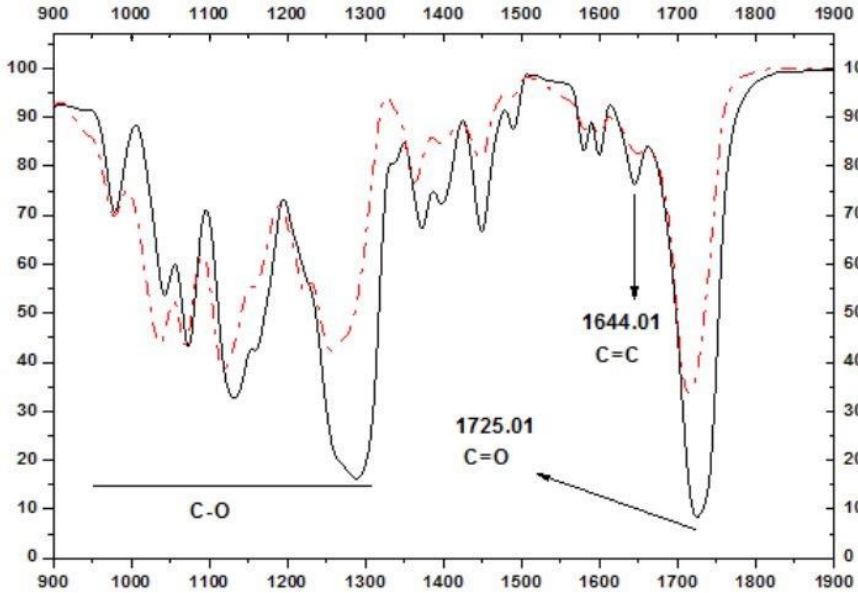


Fig. 12: ATR–FTIR spectra of liquid and solid phases of polyamides nanoparticles by solid and dashed lines, black and red, respectively, and important bonds are shown on the curve. It should be noted that and x–axis shows wavenumber (cm^{-1}) and y–axis shows intensity, respectively.

Tables' Legends:

Table. 1: Synchrotron radiation properties.

Wavelength	**10.6 µm fixed**
Output Power	**20 W**
Power Stability	**< ± 3%**
Mode Quality	**>95% TM_{00} M^2<1.3**
Beam Size	**3.8 ± 0.4 mm**

Table. 2: Position of bonds and their intensities.

Functional Group/vibration	Region cm^{-1}	Raman intensity	Infrared intensity
C=O	1680-1820	medium	Strong
C=C	1500-1900	strong	Weak
C-C	600-1300	medium	Medium
CH_3	1380	medium	Strong
CH_3 & CH_2	1400-1470	medium	Medium
C-C Aromatic	1580-1600 1450-1500	Strong medium	Medium Medium