

Enrichment of Nanodiamond Surfaces with Carboxyl Groups for Doxorubicin Loading and Release

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Abstract. In their pristine state, nanodiamond crystals produced via detonation techniques containing several functional groups present on the surface including amine, amide, alcohol, carbonyl, and carboxyl. These functional groups facilitate nanodiamond to interact drugs so as to nanodiamond is potential for medical application such as drug delivery. Even though research on the use of nanodiamond for this application has been conducted widely, research on the effect of enrichment of nanodiamond surface with carboxyl functional groups for drug loading and release has not been explored extensively. Therefore, in this paper, the effect of carboxyl-terminated nanodiamond (ND-COOH) on drug loading and release will be presented. The enrichment of nanodiamond with carboxyl groups was undertaken by treating nanodiamond with sulphuric acid and nitric acid. The results show that the doxorubicin (DOX) loading and release efficiencies of ND pristine are higher than that of ND-COOH.

1. Introduction

Nanodiamond can be synthesized by several methods such as shock wave, chemical vapour deposition (CVD), high pressure and high temperature (HPHT) and detonation. Synthesis using detonation method generates diamond with the particle size of about 4-5 nm [1]. Due to their excellent properties of biocompatibility, non-toxicity, large surface area, nitrogen vacancy, high thermal conductivity, high hardness nanodiamonds have found applications in various fields such as drug delivery, photonic devices, cellular bio-imaging and composite [2]. One of the most interesting chemical properties of nanodiamond is that its surface can be easily modified with other molecules or functional groups[3, 4] making them suitable for medical application such as drug delivery [5, 6].

Huang et al. [7] were the first to use nanodiamonds for drug delivery. They successfully loaded doxorubicin hydrochloride (DOX), one of the drugs commonly used in cancer chemotherapy, onto the surface of functionalized nanodiamond and successfully delivered the drug into human cells of colorectal carcinoma with efficacy [7]. Recently, significant efforts have been undertaken to understand and improve the chemical properties of nanodiamond surface and its effect on the efficiency of drug loading using simulation tools such as density functional-based tight binding (DFTB) and molecular dynamics (MD) [8-10]. It has been suggested that replacement of the functional groups present on the surface of nanodiamonds such as amine, amide, alcohol, carbonyl, and carboxyl with the



carboxylic acid functional group provides as a good mediator for the interaction between nanodiamond surface and drugs [10-12].

Research on the development of nanodiamond as drug carrier has been widely conducted for 5 years [13-19]; however a detailed study of the effect of nanodiamond surface enrichment with specific functional groups to the drug loading and release efficiencies of this material has not been extensively explored. Therefore, this paper presents the study of the relationship between the nanodiamond surface enriched with carboxyl groups and its ability for the drug (DOX) loading and release.

2. Experimental section

2.1 Materials

Nanodiamond purchased from Changsha 3 Better Ultra-hard Materials Co.,Ltd, China with the size of about 5-50 nm, 98% H₂SO₄, HNO₃ and aquabidest. All reagents are of analytical grades purchased from Merck Index Indonesia.

2.2 Preparation of carboxyl-enriched nanodiamond

Synthesis of ND-COOH was carried out according to the procedures in Ref. [20] with slight modification. 500 mg of ND-pristine had been heated in a mixture of strong acid (98% H₂SO₄ and 65% HNO₃ at the ratio 3 : 1) at 90 °C in water bath for 10 hours, equipped with a stirrer. To eliminate the remaining acid content, the addition of distilled water into the sample. Nanodiamond with acid separation is done by centrifugation at 6000 rpm. Subsequently, the supernatant was dried in an oven at a temperature of 35 °C for 1 hour and 80 °C for 4 hours.

2.3 Characterisation

Several characterization techniques were applied including FTIR, TEM, TGA and PSA. FTIR was performed using FTIR Prestige 21 Shimadzu, meanwhile TEM images were obtained using JEOL JEM 1400. A thermogravimetric analyzer (TGA 4000 Perkin Elmer) was used to measure the thermal weight loss of functional groups at the temperature range of 530–900 °C with heating rate of 5 °C/min under nitrogen stream.

2.4 Doxorubicin loading and release

2.4.1 Preparation of standard solution

The standard solution was prepared by dissolving doxorubicin (DOX) into aquabidest into buffer acetate (pH 7.4) at different concentrations: 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm and 35 ppm. The measurement of the absorbances of every concentration was conducted using UV-Vis spectrophotometer (UV-Vis Shimadzu UV-1201) at the wavelength of 480 nm.

2.4.2 Doxorubicin loading onto nanodiamond

Doxorubicin loading onto nanodiamond was carried out by dissolving DOX into buffer phosphate (pH 7.4) and then stirred for 30 minutes. After that, different concentrations of DOX was added 10, 20, 30, 40, 50, 60 ppm onto 0.003 g ND. The mixture was then stirred back for 1 hour. Moreover, the mixture was then centrifuged for 10 minutes and 6000 rpm in order to separate the nanodiamond loaded with DOX and unadsorbed DOX. Subsequently, DOX unadsorbed was analyzed using UV-Vis spectrophotometer at 480 nm to determine the loading DOX on ND surface.

2.4.3 Releasing of nanodiamond

Samples applied for drug *release* were ND-DOX and ND-COOH-DOX from DOX loading. The samples were re-suspended in 10 mL buffer phosphate(pH 7.4) and stirred for 30 seconds. After that, they had been incubated for 1 hour at 37°C. The next step was the samples had been stirred back for 10

seconds and centrifuged 6000 rpm for 10 minutes to separate nanodiamond and filtrate. The filtrates containing DOX were then analyzed using UV-Vis spectrophotometer at 480 nm.

3. Results and Discussion

3.1 Characterisation of nanodiamond after treatment

3.1.1 Fourier Transform InfraRed (FTIR)

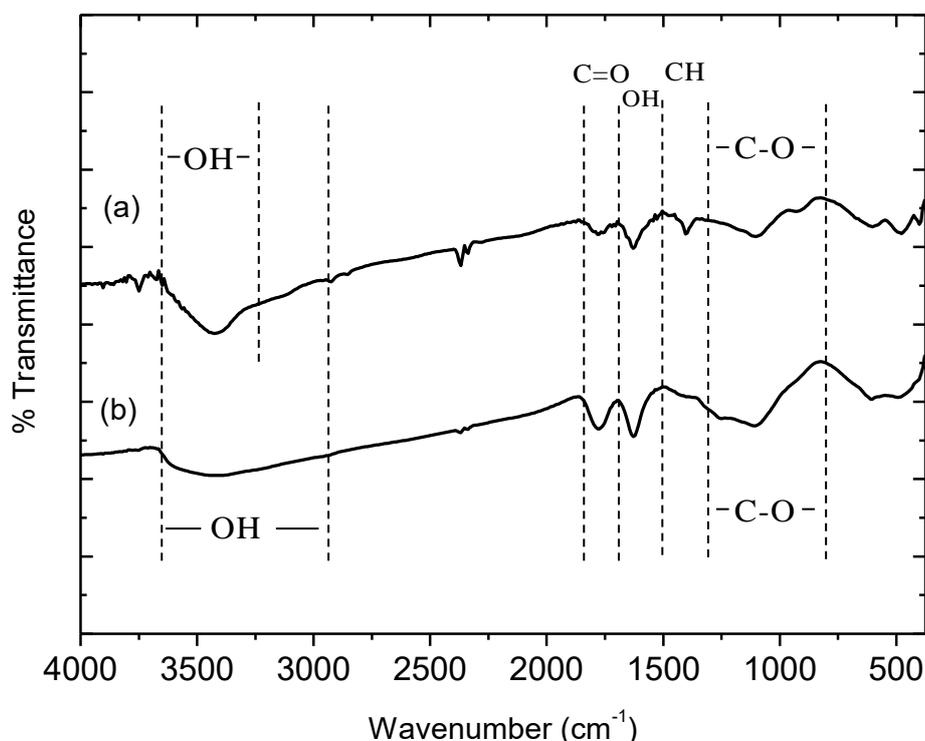


Figure 1. FTIR spectra of (a) nanodiamond untreated (top), (b) nanodiamond modify with sulphuric acid and nitric acid (bottom).

The FTIR spectra of ND-pristine in Figure 1(a) show several peaks with the intense peak observed at 1103.28 cm^{-1} indicating the presence of C-O-C vibration mode. In addition, the peaks observed at 1404.18 cm^{-1} and 2924.09 cm^{-1} are attributed to the bending vibration and overextend of C-H bond, respectively. The vibration mode at 1627.92 cm^{-1} and 3425.58 cm^{-1} are assigned to bending and stretching vibration of -OH, respectively. The presences of C=O and C-H bonds stretching vibrations were observed respectively at 1720.50 and 2924.09 cm^{-1} [21].

Figure 1(b) shows the FTIR spectra of the ND-COOH. The peaks observed at 1111.00 to 1242.16 cm^{-1} , 1627.92 cm^{-1} , 1774.51 cm^{-1} , and 3425.58 cm^{-1} indicate the presence of C-O-C bond vibration, -OH bending vibration, the vibration modes of the C=O and -OH stretching vibration, respectively [22]. These two later peaks proved the existence of the -COOH group attached onto nanodiamond surface [20]. Both samples contain -COOH functional groups; however, the treatment of pristine ND by sulphuric acid and nitric acid resulted in enrichment of ND with -COOH groups.

3.1.2 Transmission Electron Microscopy (TEM)

As seen in Figure 2(a), before treatment using H_2SO_4 and HNO_3 , the diamond nanoparticles tend to agglomerate; however, after oxidation using the mixture of both chemical reagents, the agglomerate was then separated into small clusters consisting of particles with the size of 4-5 nm which can be seen in Figure 2(c). The TEM results suggest that the use of sulphuric acid and nitric acid not only oxidized the nanodiamond surface to produce carboxyl terminated nanodiamond but also deagglomerated the nanodiamond into small clusters, even individual particles. This is required since the agglomerated nanodiamond is inappropriate for medical application especially for drug carrier [2].

As seen in Figure 2(b) and (d), the diffraction pattern shows that both samples have the crystal structure of (111), (220), and (311). This indicates that there is no change in the crystal structure of nanodiamond due to enrichment with carboxyl functional groups on its surface which is good for drug carrier application.

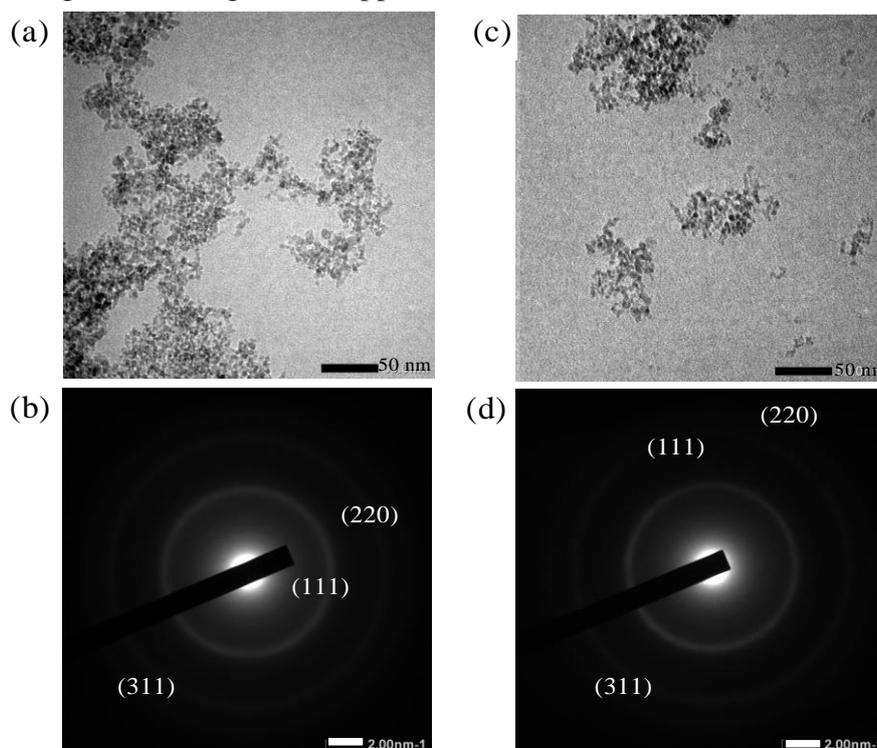


Figure 2. Bright field (BF) TEM images (upper) and diffraction pattern (lower) of as received-ND (a) (b) and of carboxyl-enriched nanodiamond (c) (d).

3.1.3 Thermo Gravimetric Analysis (TGA)

Thermograms from TGA analysis of nanodiamond before and after enrichment with carboxyl functional groups are presented in Figure 3. Thermogravimetry analysis was conducted at temperatures between 50–900 °C at a heating rate of 5 °C min⁻¹. As seen in Figure 3(a) and Table 1 for pristine nanodiamond, there is a slight weight loss (approximately 26.51%) between 70–110 °C, which can be associated with the evaporation of water due to hygroscopic hydroxyl groups. The second insignificant decline occurred at temperatures between 150–300 °C with the mass change of about 18.39%, attributed to heat-related degradation of functional groups. Meanwhile, Figure 3(b), it can be seen that there is a weight loss at the temperature between 95–220 °C (47.87%), which is much larger than that of pristine nanodiamond, indicating a much heavier mass of water associated with a carboxyl group. The second weight loss occurred between 295–450 °C, assigned to heat-related degradation of the molecule.

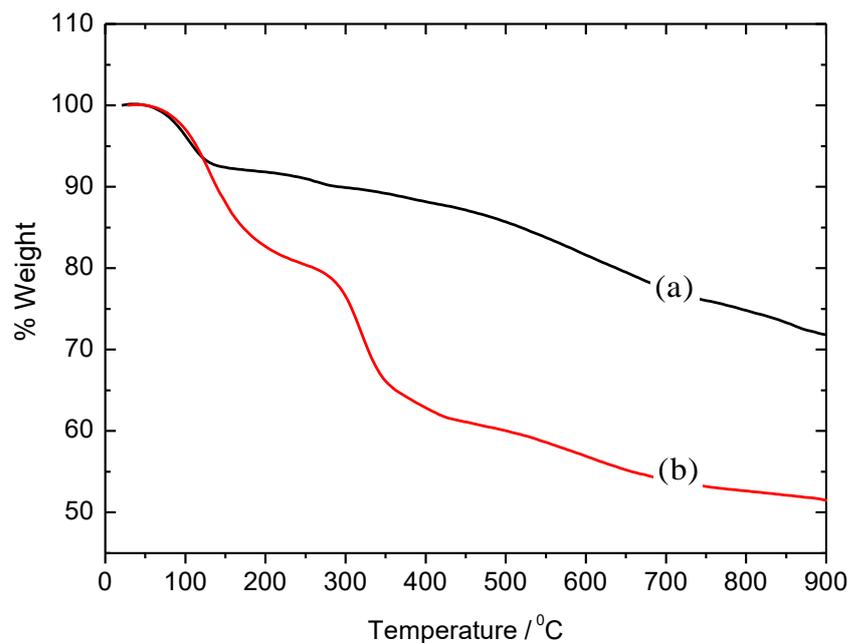


Figure 3. Thermogram of (a) as received nanodiamond and (b) carboxyl-enriched nanodiamond

Table 1. The mass change of pristine nanodiamond (ND) and carboxyl enriched nanodiamond (ND-COOH)

Samples	Onset (°C)	Mass Change (%)
ND	72.5	26.51
	256.0	18.39
ND-COOH	95.9	47.87
	298.7	31.73

3.2 DOX Loading

In order to investigate the adsorption efficiency of ND-COOH and ND, both samples were applied to adsorb DOX. The adsorption of DOX was conducted in different concentration. The results of DOX adsorption is presented in Figure 5 and Table 3. The results show that after being modified with sulphuric acid and nitric acid, the adsorption efficiency of ND-COOH is higher than that of ND. Therefore, it can be said that enrichment of nanodiamond surfaces with carboxyl groups enables nanodiamond to adsorb DOX more efficiently.

DOX loading on both pristine and COOH-enriched nanodiamonds at different concentration is presented in Figure 4. It can be seen that the higher the concentration of DOX, the higher DOX loaded on both samples. However, DOX loaded on ND pristine is little higher than on N-COOH. This difference could be due to the distinction of the functional groups present on particle surfaces of both samples which influence the interaction between DOX and nanodiamond surfaces.

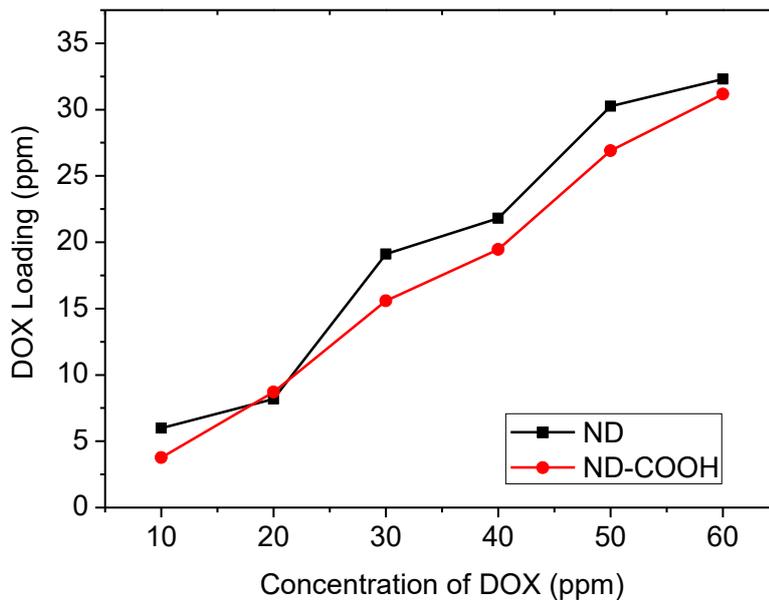


Figure 4. DOX loading on ND and ND-COOH in different concentrations

3.3 DOX Release

DOX release from both ND pristine and ND-COOH at two different time intervals is presented in Figure 6 for 60 ppm DOX loading. It can be observed from Figure 5 that after one hour the drug release from the pristine ND was ~14% more than the treated ND, whereas after two hours the pristine ND had ~55% more drug release as compared to treated ND. This result shows that DOX is more strongly bonded to the pristine ND than ND-COOH groups of the treated ND. As mentioned previously, the pristine nanodiamond surfaces contain several functional groups such as hydroxyl, carboxyl, ether, carbonyl, etc.[2] while in carboxyl-enriched nanodiamond, nanodiamond surfaces mainly contain carboxyl groups. Therefore, this difference may influence the interaction between DOX and nanodiamond surfaces.

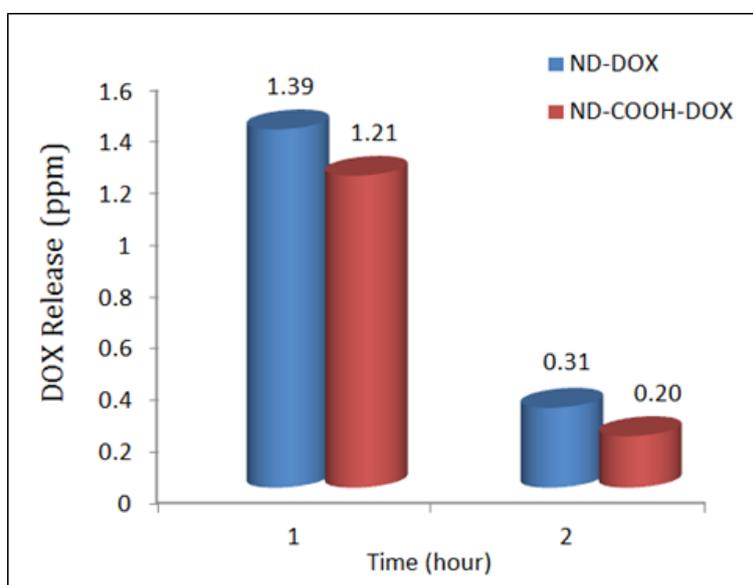


Figure 5. DOX release from pristine ND and ND-COOH

4. Conclusion

Nanodiamond without surface modification shows better loading and release efficiencies to the doxorubicin than that of nanodiamond surface enriched with a carboxyl group. The difference on the functional groups contained on nanodiamond surfaces in both samples is the main reason of the interaction between DOX and nanodiamond surface. Pristine nanodiamond contains several functional groups, namely, -OH, -COOH, -CO, etc. On the other hand, carboxyl-enriched nanodiamond, its surface contains mostly carboxyl group. The interaction energy between DOX and functional group contained on nanodiamond surface should be determined in order to convince this argument. The computational calculation of the interaction energy is on going.

References

- [1] Shenderova O A and Gruen D M, *Ultrananocrystalline Diamond*. William Andrew Publishing, Norwich, New York. 2006.
- [2] Mochalin V N, Shenderova O, Ho D and Gogotsi Y 2012 The properties and applications of nanodiamonds *Nat. Nanotechnol.* **7** 11-23.
- [3] Astuti Y, Poolton N R J and Šiller L 2013 Raman and Photoluminescence Spectroscopic Study of 1-Undecene Functionalized Nanodiamonds *J K-Theory* **1** 1597.
- [4] Astuti Y, Poolton N R J, Butenko Y V and Šiller L 2014 Evaporation and alignment of 1-undecene functionalised nanodiamonds *J Lumin* **156** 41-48.
- [5] El-Say K M 2011 Nanodiamond as a drug delivery system: Applications and prospective *J Appl Pharm Sci* **1** 29-39.
- [6] Zhu Y, Li J, Li W, Zhang Y, Yang X, Chen N, Sun Y, Zhao Y, Fan C and Huang Q 2012 The biocompatibility of nanodiamonds and their application in drug delivery systems *Theranostics* **2** 302-312.
- [7] Huang H, Pierstorff E, Osawa E and Ho D 2007 Active nanodiamond hydrogels for chemotherapeutic delivery *Nano Lett.* **7** 3305-3314.
- [8] Barnard A S 2008 Self-assembly in nanodiamond agglutinates *J. Mater. Chem.* **18** 4038-4041.
- [9] Lai L and Barnard A S 2012 Interparticle Interactions and Self-Assembly of Functionalized Nanodiamonds *J Phys. Chem. Lett.* **3** 896-901.
- [10] Kim H, Man H B, Saha B, Kopacz A M, Lee O-S, Schatz G C, Ho D, Liu W K 2012 Multiscale simulation as a framework for the enhanced design of nanodiamond-polyethylenimine-based gene delivery *J. Phys. Chem. Lett.* **3** 3791-3797.
- [11] Chow E K, Zhang E Q, Chen M, Lam R, Robinson E, Huang H J, Schaffer D, Osawa E, Goga A and Ho D 2011 Nanodiamond therapeutic delivery agents mediate enhanced chemoresistant tumor treatment *Sci. Transl. Med.* **3** 73ra21.
- [12] Schmidlin L, Pichot V, Comet M, Josset S, Rabu P and Spitzer D 2012 Identification, quantification and modification of detonation nanodiamond functional groups *Diam. Relat. Mater.* **22** 113-117.
- [13] Chen M, Pierstorff E D, Lam R, Li S-Y, Huang H, Osawa E and Ho D 2009 Nanodiamond-mediated delivery of water-insoluble therapeutics *ACS Nano* **3** 2016-2022.
- [14] Li Y, Zhou X, Wang D, Yang B and Yang P 2011 Nanodiamond mediated delivery of chemotherapeutic drugs *J. Mater. Chem.* **21** 16406-16412.
- [15] Zhang X, Wang S, Fu C, Feng L, Ji Y, Tao L, Li S and Wei Y 2012 PolyPEGylated nanodiamond for intracellular delivery of a chemotherapeutic drug *Polym. Chem.* **3** 2716-2719.
- [16] Xiao J, Duan X, Yin Q, Zhang Z, Yu H and Li Y 2013 Nanodiamonds-mediated doxorubicin nuclear delivery to inhibit lung metastasis of breast cancer *Biomaterials* **34** 9648-9656.
- [17] Li Y, Tong Y, Cao R, Tian Z, Yang B and Yang P 2014 In vivo enhancement of anticancer therapy using bare or chemotherapeutic drug-bearing nanodiamond particles *Int. J. Nanomed.* **9** 1065-1082.

- [18] Man H B, Kim H, Robinson E, Liu W K, Chow E K and Ho D 2014 Synthesis of nanodiamond-daunorubicin conjugates to overcome multidrug chemoresistance in leukemia *Nanomed.-Nanotechnol.* **10** 359-369.
- [19] Mochalin V N, Pentecost A, Li X-M, Neitzel I, Nelson M, Wei C, He T, Guo F and Gogotsi Y 2013 Adsorption of drugs on nanodiamond: Toward development of a drug delivery platform *Mol. Pharm.* **10** 3728-3735.
- [20] Jee A Y and Lee M 2009 Surface functionalization and physicochemical characterization of diamond nanoparticles *Curr. Appl. Phys.* **9** e144-e147.
- [21] Larionova I, Kuznetsov V, Frolov A, Shenderova O, Moseenkov S and Mazov I 2006 Properties of individual fractions of detonation nanodiamond *Diam. Relat. Mater.* **11–12** 1804-1808.
- [22] Lau X C, Desai C and Mitra S 2013 Functionalized nanodiamond as a charge transporter in organic solar cells *Sol. Energy* **91** 204-211.

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