Langmuir
Article
pubs.acs.org/Langmuir

Covalent Immobilization of Protein onto a functionalized Hydrogenated Diamond-like Carbon Substrate

Hari Shankar Biswas,[†] Jagannath Datta,[†,‡] D. P. Chowdhury,[‡] A. V. R. Reddy,[§] Uday Chand Ghosh,[∥] Arvind Kumar Srivastava,[#] and Nihar Ranjan Ray*[,†]

[†]*Nanocrystalline Diamond like Carbon Synthesis Laboratory, Saha Institute of Nuclear Physics, 1/AF, Bidhan Nagar, Kolkata-700 064, India,* [‡]*Analytical Chemistry Division, Bhabha Atomic Research Centre, Variable Energy Cyclotron Centre, 1/AF, Bidhan Nagar, Kolkata-700 064, India,* [§]*Analytical Chemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai-400 085, India,* [∥]*Department of Chemistry, Presidency College, 86/1 College Street, Kolkata-700 073, India, and* [#]*Electron Microscopy Laboratory, ISUD, INDUS-I, Raja Ramanna Center for Advanced Technology, Indore-452013, India*

Received June 8, 2010. Revised Manuscript Received September 24, 2010

Hydrogenated diamond-like carbon (HDLC) has an atomically smooth surface that can be deposited on high-surface area substrata and functionalized with reactive chemical groups, providing an ideal substrate for protein immobilization. A synthetic sequence is described involving deposition and hydrogenation of DLC followed by chemical functionalization. These functional groups are reacted with amines on proteins causing covalent immobilization on contact. Raman measurements confirm the presence of these surface functional groups, and Fourier transform infrared spectroscopy (FTIR) confirms covalent protein immobilization. Atomic force microscopy (AFM) of immobilized proteins is reproducible because proteins do not move as a result of interactions with the AFM probe-tip, thus providing an advantage over mica substrata typically used in AFM studies of protein. HDLC offers many of the same technical advantages as oxidized graphene but also allows for coating large surface areas of biomaterials relevant to the fabrication of medical/biosensor devices.

1. Introduction

The immobilization of biomolecules[1−6] onto surfaces is important in many fields of biological and physical sciences, including cell and molecular biology, analytical chemistry, medical diagnostics, tissue engineering, and bioprocess engineering. Since the discovery of the many advantageous properties of immobilized biomolecules, the challenge in this area has been to develop new substrate materials with appropriate structures, compositions, surface morphology and functionality to widen the understanding of immobilized biomolecules. Recently, along with the development of nanostructured materials, a range of nanomaterials with different surface properties have been utilized as substrates for biomolecule immobilization.[7−9] In particular, oxidized graphene has been demonstrated as a well-defined, ordered, and atomically smooth surface. Graphene oxide (GO)[10] is in the form of aqueous suspension of particles whose specific surface area is typically ∼72 nm^2/g. The intrinsic oxygen-containing functional groups, viz., −COOH, −OH, and so forth, as required for covalent interaction with the amine groups of proteins, are present over the surface of GO; although this covalent interaction usually happens very slowly.[11] Besides these, the surface of GO is negatively charged in the aqueous solution with a pH range from 4 to 11; thus, in buffer solutions with a pH range of 4.8−7.2, the positively charged horseradish peroxidase (HRP)[10] interacts with the negatively charged GO by electrostatic interaction, while in the buffer solutions from pH 7.2 to 8.8, HRP and GO both are negatively charged, and will repel each other. Hence immobilization of biomolecules, HRP onto GO,[10] is influenced by both covalent interaction as well as electrostatic interaction. Diamond-like carbon (DLC) can be deposited, in thin film form, in an ordered hexagonal array and subsequently hydrogenated (HDLC)[12,13] to produce an ordered structure, having a specific surface area larger than that of the GO particle by several orders of magnitude. The intrinsic hydrogen-containing functional groups, viz., $=CH_2$, sp^2 carbons (C=C) etc. are present over the HDLC surface, which is in the neutral charge-state in the presence of buffer solution of pH 8.5. We show here that biomolecules can be immobilized onto the HDLC surface having high loading capacity of proteins with no conformal change and having covalent interaction. Also we show that the covalent interaction between reactive functional groups in the HDLC surface and the amine group in the 3,4-dihydroxy-L-phenylalanine (DOPA)[14] should be effectively much stronger than that between reactive functional groups in the HDLC surface and the amine groups in the bovine serum albumin (BSA) proteins. Thus the experimental results in the present work will show the necessity for a DOPA-modified HDLC

*Corresponding author. E-mail: niharranjan.ray@saha.ac.in.

(1) Lee, H; Rho, J; Messersmith, P. B. *Adv. Mater.* **2009**, *21*, 431–434.
(2) MacBeath, G. *Nat. Biotechnol.* **2001**, *19*, 828–829.
(3) Hodneland, C. D.; Lee, Y. D.; Mrksich, M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5048–5052.
(4) Zhang, G; Suggs, L. J. *Adv. Drug Delivery Rev.* **2007**, *59*, 360–373.
(5) Chen, H; Hsieh, Y. *Biotechnol. Bioeng.* **2005**, *90*, 405–413.
(6) Nebel, C. E.; Shin, D.; Rezek, B.; Tokuda, N.; Uetsuka, H.; Watanabe, H. *J. R. Soc. Interface* **2007**, *4*, 439–461.
(7) Kim., J.; Grate, J. W.; Wang, P. *Chem. Eng. Sci.* **2006**, *61*, 1017–1026.
(8) Zhi., C.; Bando, Y.; Tang, C.; Golberg, D. *J. Phys. Chem. B* **2006**, *127*, 17144–17145.
(9) Tsang, S. C.; Yu, C. H.; Gao, X; Tam, K. *J. Phys. Chem. B* **2006**, *110*, 16914–16922.
(10) Zhang, J.; Zhang, F.; Yang, H.; Huang, X.; Liu, H.; Zhang, J.; Guo, S. *Langmuir* **2010**, *26*(9), 6083–6085.
(11) Bornscheuer, U. T. *Angew. Chem., Int. Ed.* **2003**, *42*, 3336–3337.
(12) Ray, N. R. Iyengar, A. N. S. In *Proceedings of the Sixth International Conference on Reactive Plasmas and 23rd Symposium on Plasma Processing* (*ICRP-6-(SPP-23)*); Hatakeyama, R, Samukawa, S., Eds.; Organizing Committee of ICRP-6/SPP-23: Dept. of Electrical Engineering, Tohoku University, Sendai, Japan, 2006, pp 583−584.
(13) Singha, A.; Ghosh, A.; Ray, N. R.; Roy, A. *J. Appl. Phys.* **2006**, *100*(8), 44910.
(14) Waite, J. H.; Tanzer, M. L. *Science* **1981**, *212*, 1038–1040.

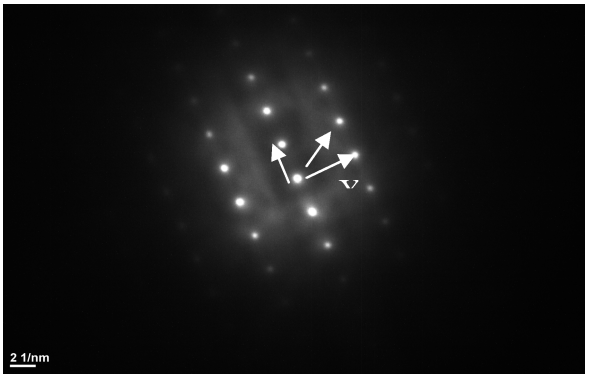


Figure 1. Typical electron diffraction pattern taken by TEM from plan view of the HDLC sample with the zone axis along the [110] direction.

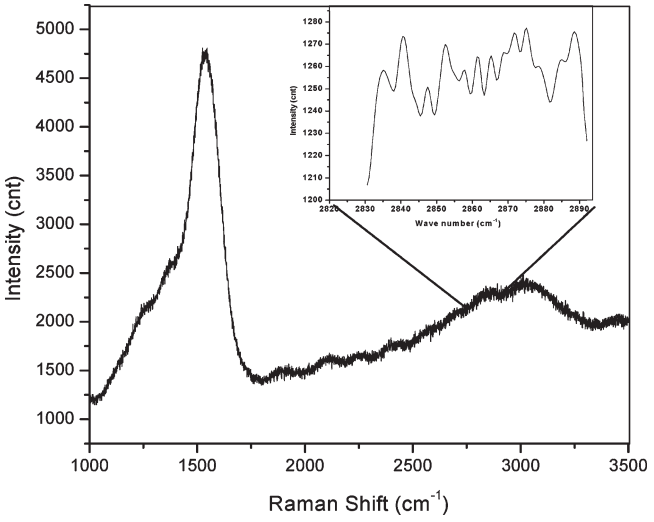


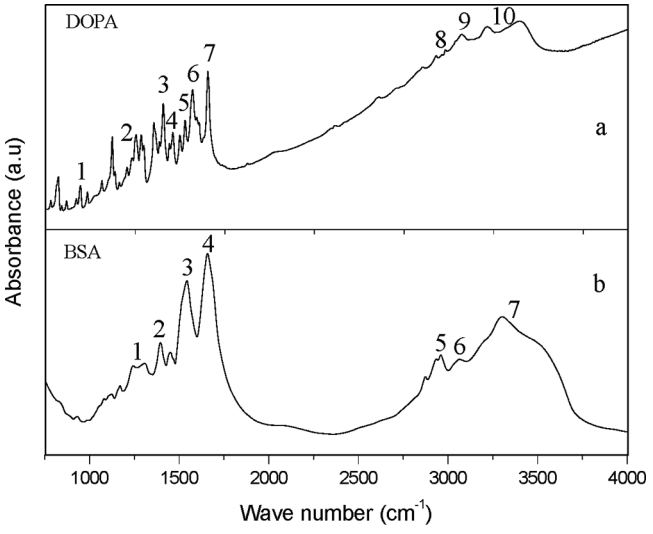
Figure 2. Raman spectrum of HDLC film onto Si (1 0 0) substrate; Excitation wavelength 488 nm, laser power 7.5 mW, grating 1800 gr/mm, objective 100×, aperture 100 μm. The inset gives the expanded spectral region of 2820−2950 cm^{-1}, where the vibrational modes of CH, CH_2, and CH_3 are observed.

surface in order to immobilize BSA protein onto it through covalent interaction, having a better benefit compared with other substrates such as GO particles.

2. Materials and Methods

2.1. Materials. Tris (hydroxymethyl) aminomethane ($C_4H_{11}NO_3$; M.W. 121.14; 99.9%, Sisco Research Laboratories Pvt. Ltd.), DOPA (99%, Sisco Research Laboratories Pvt. Ltd.), BSA (96.96%, Sisco Research Laboratories Pvt. Ltd.), and p-type mirror polished Si (100) wafer were used in the experiment. Ultrapure water using the Milli-Q system of Millipore Co. was used throughout the experiment.

2.2. Preparation of HDLC Thin Film Deposited on Si (100) wafer. A straightforward synthesis of HDLC by the low-pressure biased enhanced nucleation (BEN) process[15] at room temperature in an asymmetrically capacitively coupled radio-frequency (RF; 13.56 MHz) device,[12] involves the following two steps: (1) etching of mirror polished Si (100) substrate of 10 mm diameter for 15 min in a pure hydrogen (flow rate ∼ 500 sccm) plasma, at a pressure of 0.193 mbar, produced by 30 W RF power producing dc self-negative bias (approximately −200 V), to remove an oxide layer from the surface of Si (100) and (2) in situ BEN process[15] using He (flow rate ∼ 1500 sccm) plasma produced by 50 W RF power producing dc self-negative bias (approximately −200 V), with H_2 (flow rate ∼ 500 sccm) and CH_4 (flow rate ∼ 50 sccm) gases at a total pressure of 0.756 mbar and at substrate temperature ∼14 °C, for 30 min deposition time.



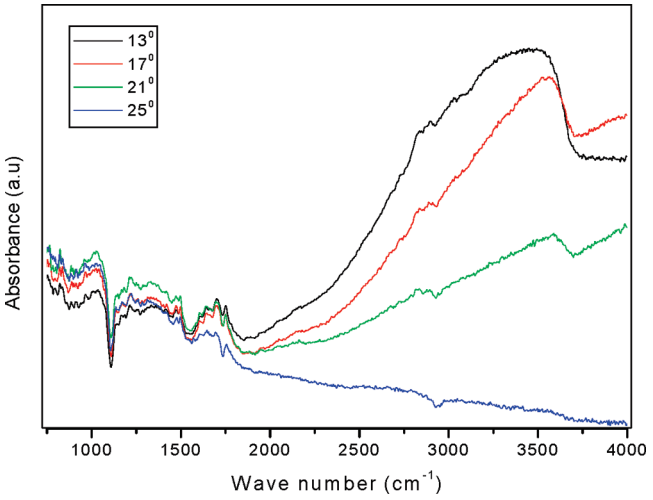


Figure 3. (a,b) Typical FTIR spectrum of DOPA and BSA powder in a KBr pellet. (c) Typical FTIR spectra of BD-HDLC with variable angles 13°, 17°, 21°, and 25°.

Table 1. Tentative Assignments of Peaks in the FTIR Spectra of DOPA Powder

peak no.	wavenumber (cm^{-1})	tentative assignment
1	820−944	ring vibration
2	1122−1285	C−N stretching
3	1408	COO-symmetric stretching
4	1460	CH_2 stretching
5	1528	C=C ring stretching
6	1571	COO-asymmetric stretching
7	1658	N−H bending
8	2850−2982	aliphatic C−H stretching
9	3072	aromatic C−H stretching
10	3100−3400	N−H bending

2.3. Immobilization of Protein onto the Surface of HDLC. Phosphate (0.2 M) and 10 mM Tris buffers of pH 7.8 and 8.5 respectively were prepared at room temperature. A solution was prepared by adding 50 mg of DOPA in 25 mL of 10 mM Tris

(15) Robertson, J.; Gerber, J.; Sattel, S.; Weiler, M.; Jung, K.; Ehrhardt, H. *Appl. Phys. Lett.* **1995**, *66*(24), 3287–3289.

(pH 8.5). The HDLC sample, cleaned by isopropyl alcohol and rinsed in ultra pure water, was immersed in the DOPA solution for 2 h at room temperature, under continuous stirring conditions using a magnetic stirrer. The DOPA-treated HDLC (D-HDLC) film was rinsed by ultrapure water and dried with nitrogen and was further treated with a BSA solution, prepared by dissolving 250 mg of BSA in 25 mL of 0.2 M phosphate buffer, for 2 h at room temperature under slow stirring. The BSA-treated D-HDLC sample (BD-HDLC) was rinsed with ultra pure water and dried with nitrogen. Similarly, the HDLC sample was treated with BSA solution only.

Table 2. Tentative Assignments of Peaks in the FTIR Spectra of BSA Powder

peak no.	wavenumber (cm^{-1})	tentative assignment
1	1200−1290	C−N stretching, C−H bending
2	1470−1485	C−N stretching, C−H bending
3	1531	amide II band
4	1652	amide I band
5	2825−3000	aliphatic and aromatic C−H stretching, broad O−H stretching
6	3063	amide A (mainly −NH stretching vibration)
7	3400	broad band for O−H/N−H stretching and H-bonding

2.4. Characterization of Samples. Transmission electron microscopy (TEM) used for crystalline characterization of our HDLC sample was performed using a Philips CM200 kV TEM machine operated at 200 kV with LaB_6 filament. For the plan view by TEM, the sample was prepared by back-side thinning of Si substrate by dimpling and ion milling, whereas the sample for cross-sectional TEM was prepared by cutting the substrate into two equal sizes and then inserting them in the slot made in a brass

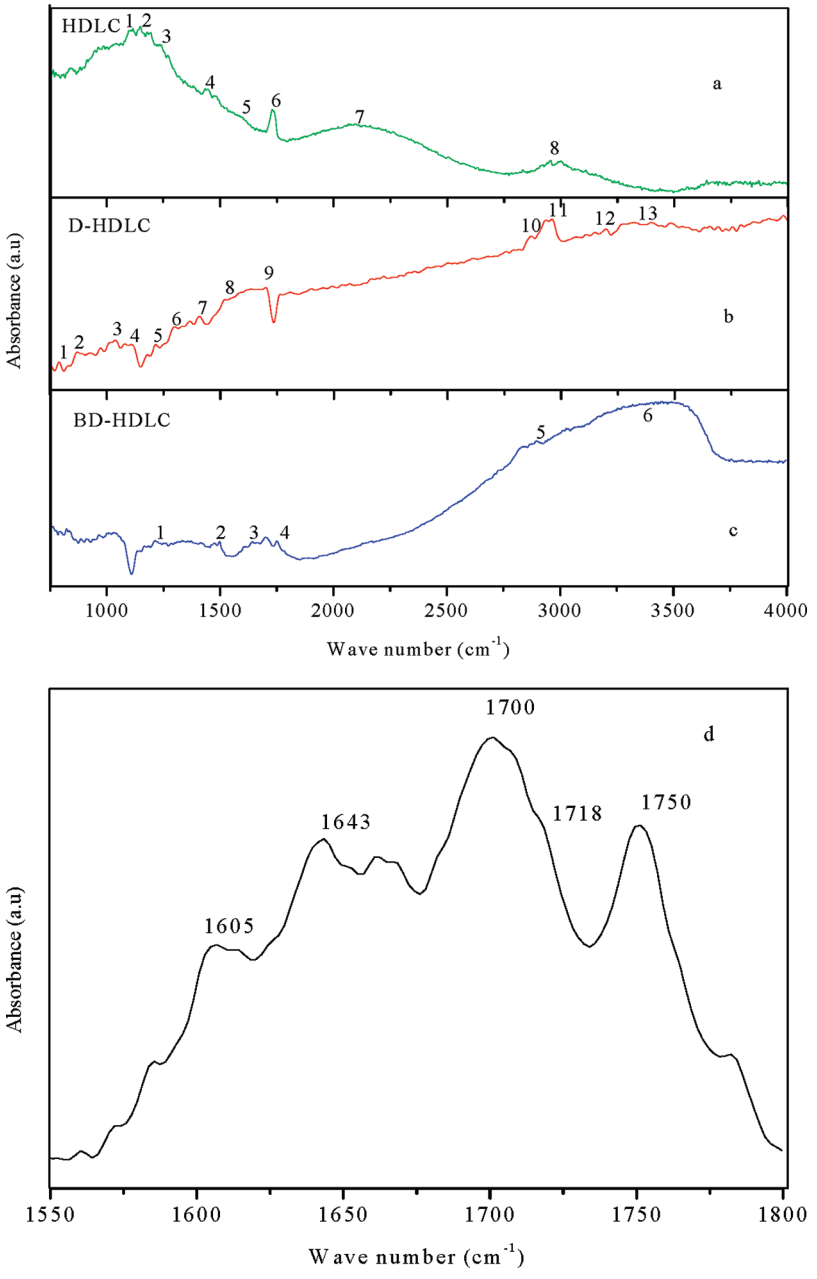


Figure 4. (a) Typical FTIR spectra at an angle of 13° of the HDLC sample surface. (b) Typical FTIR spectra at an angle of 13° of the D-HDLC sample surface. (c) Typical FTIR spectra at an angle of 13° of the BD-HDLC sample surface. (d) Typical FTIR spectra at an angle of 13° of the amide-I band present in panel c.4c.

Table 3. Tentative Assignments of Peaks in the FTIR Spectra of the HDLC Surface

peak no.	wavenumber (cm-[1])	tentative assignment
1	1130	sp^2 CH_2
2	1180	sp^3 CH_2
3	1250	sp^3 CH
4	1450	sp^2-Sp^3
5	1615	sp^2 C═C stretching
6	1735	C═O
7	2000−2300	C≡C stretching
8	2850−3200	sp C−H and sp^2 C−H

rod. Finally, the rods containing Si were inserted into a brass tube. Many slices were cut from the brass tube. Each slice was mechanically polished, dimpled, and ion milled. For the structural characterization of the HDLC sample, a Raman spectrum was obtained by a confocal Micro Raman spectrometer (LabRAM *HR* Vis, Horiba Jobin Yvon SAS France), which includes a 800 mm focal length Czerny−Turner-type spectrograph equipped with mirrors (reflective optics) having high spectral resolution of 0.1 cm^{-1}/pixel at 488 nm with a 1800 gr/mm grating. For determining the reactive chemical functional groups present in the sample, the Fourier transform infrared (FTIR) spectrum of the sample was obtained by an Infrared Fourier Vacuum Spectrometer Vertex 70v (Bruker Optik GmbH Germany) with fixed, horizontal IR transmission through DOPA and BSA protein powder samples in KBr pellet respectively and variable angle (13−85°) reflection with ATR crystal (Zn−Se) for solid HDLC samples. KBr beam splitter, DLaTGS detector, MIR source, Rock Solid Interferometer system, spectral resolution (> 0.4 cm^{-1}) and contamination free vacuum (< 0.2 mbar) are the key technical features of Vertex 70v. An atomic force microscope (AFM) topography image of the surface of the sample was obtained in noncontact mode by a Multimode Scanning Probe Microscope (Agilent AFM 5500 series, U.S.A.) having a multipurpose small scanner with a low coherence laser (1 mW power, 670 nm wavelength, coherence length (< 50 μm), scan range: XY: 0−10 μm ; Z: 0−2 μm, noise level: $XY < 0.1$ nm rms, $Z < 0.02$ nm rms. Surface energy of the HDLC sample was measured by a ramé-hart model 250 standard goniometer with DROPimage Advanced v2.4 of ramé-hart instrument co., U.S.A.

3. Results and Discussion

Figure 1 shows the electron diffraction pattern taken from plan view of the HDLC sample. It shows single crystal pattern indexed with the zone axis along the [110] direction. The pattern was indexed with hexagonal lattice parameters $a = 2.62$ Å and $c = 6.752$ Å. The electron diffraction pattern indexed with different orientation gives the same hexagonal lattice parameters, which are different from the hexagonal graphene/graphite structure. From the cross-sectional TEM measurement the typical thickness of the HDLC sample is 168 ± 5 nm. Assuming the density of the HDLC material[16] is ∼3 g/cm^3, the diameter of the HDLC sample being 1 cm, the typical estimated value of the deposited mass of HDLC is 40 μg; hence the specific surface area ∼10^{11} nm^2/g of our HDLC material is several orders of magnitude higher than that of GO particles.

The Raman spectrum (Figure 2) is composed of broad bands centered at approximately 1535 cm^{-1} (G band), 1369 cm^{-1} (D band), and 2850 cm^{-1} (vibrational modes of CH, CH_2, and CH_3 groups). The G band corresponds to the symmetric E_{2g} C═C stretching mode in sp^2 bonding in carbon related materials, while the D band is attributed to bond angle disorder in the graphite-like microdomains affected by sp^3 bonds.[13,17] The vibrational modes[13,17,18] of C═C, CH, CH_2, and CH_3 functional groups present in the HDLC sample indicate the possibility of covalent bonding by them with the suitable functional groups.

Table 4. Tentative Assignments of Peaks in the FTIR Spectra of the D-HDLC Surface

peak no.	wavenumber (cm^{-1})	tentative assignment
1	788	C−C skeletal stretching
2	865	C−H bending (out of plane)
3	1030	C−C stretching
4	1080	C−C−N stretching
5	1227	C−C−H bending
6	1304	CH_2 wagging
7	1408	COO^- symmetrical stretching
8	1510	NH_3^+ symmetrical bending
9	1685	C═O stretching
10	2864	C−H stretching
11	2960	CH_2 asymmetric stretching
12	3060−3190	C−H stretching (aromatic)
13	3300−3500	O−H stretching with H-bonded

Table 5. Tentative Assignments of Peaks in the FTIR Spectra of the BD-HDLC Surface

peak no.	wavenumber (cm^{-1})	tentative assignment
1	1220	C−N stretching, C−H bending
2	1470−1485	C−N stretching, C−H bending
3	1600−1700	amide I band
4	1753	first overtone of O−H
5	2825−3050	aliphatic and aromatic C−H stretching, broad O−H stretching
6	3100−3600	broad band for O−H/N−H stretching and H-bonding

The surface energy of the HDLC sample, as measured by a goniometer, is approximately 39.6 ± 0.02 mJ/m^2, which shows that the surface of the HDLC sample is hydrophobic. So there is a possibility of protein adsorption due to hydrophobic interaction.[19,20] Electrochemical measurements in the Tris buffer (pH 8.5) solution indicate that the surface of the HDLC sample is electrically neutral; hence there is no possibility of protein adsorption due to electrostatic interaction.

Figure 3a,b shows the typical FTIR spectrum (absorbance vs wavenumber) of DOPA and BSA protein. The FTIR spectra of DOPA (Figure 3a) and BSA protein (Figure 3b) indicate the presence of functional groups in the samples respectively before their use for modification of the surface of the HDLC sample.

Tentative assignments of peaks in the FTIR Spectra of DOPA and BSA powder are given in Tables 1 and 2, respectively.

Figure 3c shows the typical FTIR spectrum (absorbance vs wavenumber) of the BD-HDLC with variable angle of incidence (reflection) using Zn−Se ATR crystal. Since the intensities of the characteristic peaks in the spectrum are prominent at an angle of 13°, we have analyzed all the experimental results on the FTIR spectrum at this particular angle.

Figure 4a−c shows the typical FTIR spectrum of HDLC, D-HDLC, and BD-HDLC samples, respectively. Characteristic peaks of the functional groups present in the HDLC, D-HDLC, and BD-HDLC samples, as shown in Figure 4a−c, are assigned[13,21−24] and are given in Tables 3, 4 and 5, respectively.

(16) Staryga, E.; Bak, G. W. *Diamond Relat. Mater.* **2005**, *14*, 23–24.
(17) Casiraghi, C.; Ferrari, A. C.; Robertson, J. *Phys. Rev. B* **2005**, *72*(14), 85401.
(18) Okabayashi, H.; Kitagawa, T. *J. Phys. Chem.*. **1978**, *82*(16), 1830–1836.
(19) Whitesides, G. M.; Grzybowski, B. *Science* **2002**, *295*, 2418–2421.
(20) Kokkoli, E.; Zukoski, C. F. *Langmuir* **1998**, *14*, 1189–1195.
(21) Tao, C; Yang, S; Zhang, J; Wang, J. *Appl. Surf. Sci.* **2009**, *256*, 294–297.
(22) Ramachandran, E.; Natarajan, S. *Cryst. Res. Technol.* **2007**, *42*(6), 617–620.

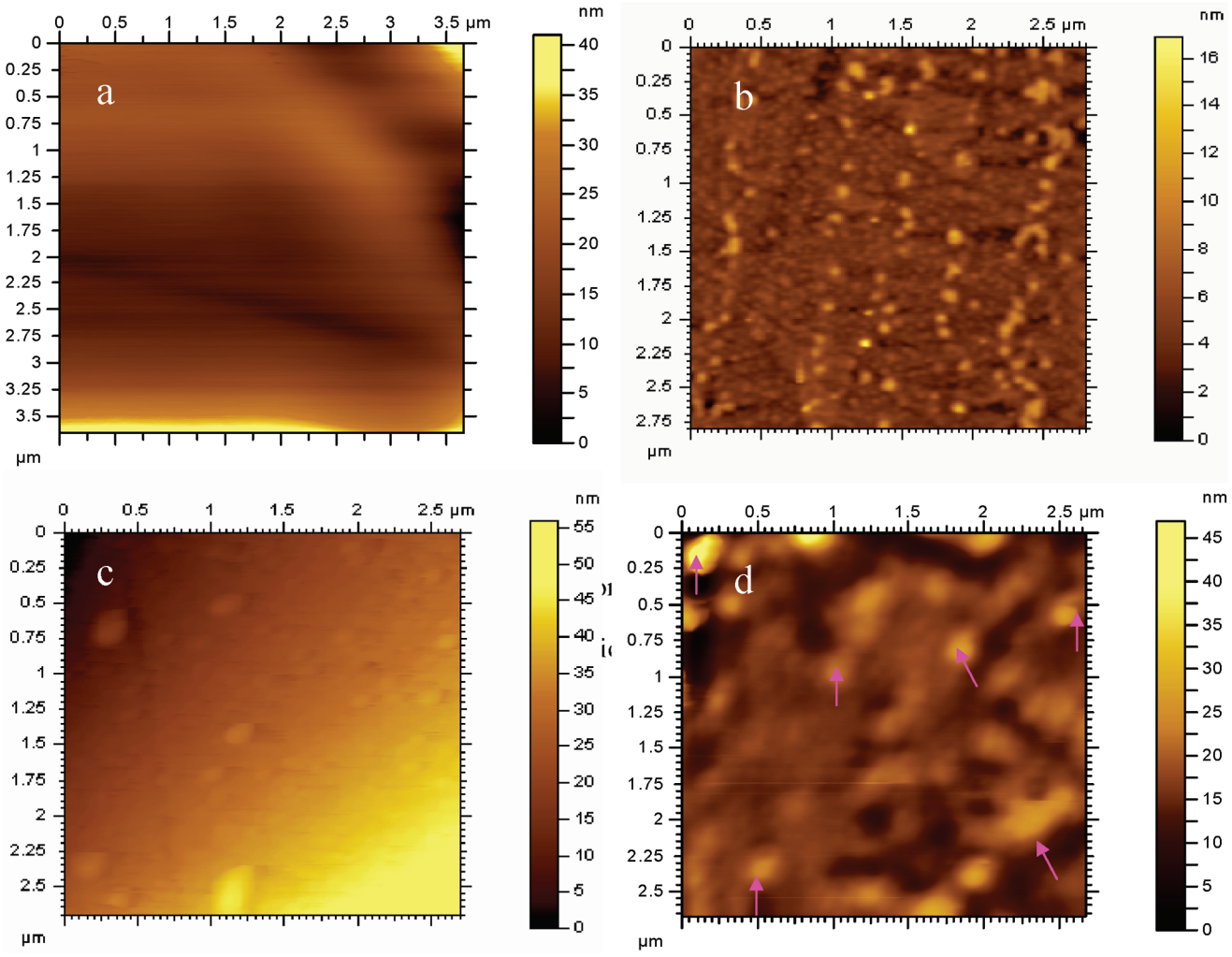


Figure 5. Non-contact mode AFM topography images of (a) HDLC surface: scan size 3.5 μm × 3.5 μm; (b) D-HDLC surface: scan size 2.5 μm × 2.5 μm; (c) BSA-treated HDLC surface: scan size 2.5 μm × 2.5 μm; and (d) BD-HDLC surface: scan size 2.5 μm × 2.5 μm.

The FTIR spectrum of the D-HDLC sample (Figure 4b) is very different from that of DOPA (Figure 3a) and HDLC (Figure4a), respectively. It seems that many of the functional groups in the DOPA (Figure 3a) and HDLC (Figure 4a) participated in covalent interactions, producing the resultant functional groups as observed in Figure 4b. The necessary functional group for immobilization of protein is amine (NH_2/NH/N). The functional group amine (NH_2/NH/N), present in the D-HDLC sample, is observed in the FTIR spectrum of the sample (Figure 4b). From the tentative assignment of the peaks (Table 4) in the spectrum using various literature procedures (or data)[24−27] it is plausible to say that the HDLC sample is functionalized with secondary amine via covalent interactions between various vibrational modes, such sp^2 (C═C) carbon atoms, hydrogenated carbon atoms ═CH_2, −CH_3, and so forth, having ordered structure in the HDLC sample and primary amine as present in the DOPA sample. The FTIR spectrum of the D-HDLC sample is clearly different from that of the BD-HDLC sample as evident from Figure 4b,c. From the tentative assignment of peaks (Table 5), it is evident that the BD-HDLC sample contains an amide-I band, as explicitly shown in Figure4d.

Amide-I band components (Figure 4d) result from antiparallel pleated sheets and β turns of proteins.[24] The band also contains α helical structures (~ 1655 cm^{-1}) and β-pleated sheet structures (~ 1655 cm^{-1}).[24] This result indicates retention of the conformation of immobilized protein onto the ordered structure of carbon atoms (HDLC) due to covalent bonding. The amide-II band (~1531 cm^{-1}) as present in the BSA protein (Figure 3b) is absent in the spectrum of BD-HDLC (Figure 4c). Thus it is possible that amide-II (−NH bending vibrations) band participates in the covalent interaction between BSA protein and D-HDLC samples, resulting in the BD-HDLC sample. The AFM image (Figure 5a) shows topography of the surface of HDLC sample; its roughness value (~0.05 nm) shows an atomically smooth surface. The AFM images (Figure 5b,c,d) show the topography of the surface of D-HDLC, BSA-treated HDLC surface, and BD-HDLC samples, respectively. Their roughness value (~0.1 nm) is higher, by an order of magnitude, than that of the bare surface of HDLC. The immobilized proteins are globular in nature and they are aggregated as marked by arrows.

It is observed that there are a few immobilized proteins on the BSA-treated HDLC surface (Figure 5c) but a large number of

(23) Martinu, L.; Rave, A.; Boutard, D.; Houle, S.; Poitras, D.; Vella, N.; Wertheimer, M. R. *Diamond Relat. Mater.* **1993**, *2*, 673–677.
(24) Stuart, B. *Biological Applications of Infrared Spectroscopy*; Wiley: UK, 1997; pp 113−150.
(25) Kalsi, P. S. *Spectroscopy of Organic Compounds*, 6th Ed. New Age Int. (P) Ltd.: New Delhi, 2004, pp 65−177.
(26) Pavia, D. L.; Lampman, G. M.; Kriz, G. S. *Introduction to Spectroscopy*, 4th ed. Harcourt Brace & Company: Orlando, FL, 2009; pp 30−76.
(27) Tencer, M.; Charbonneau, R.; Lahoud, N.; Berini, P. *Appl. Surf. Sci.* **2007**, *253*, 9209–9214.

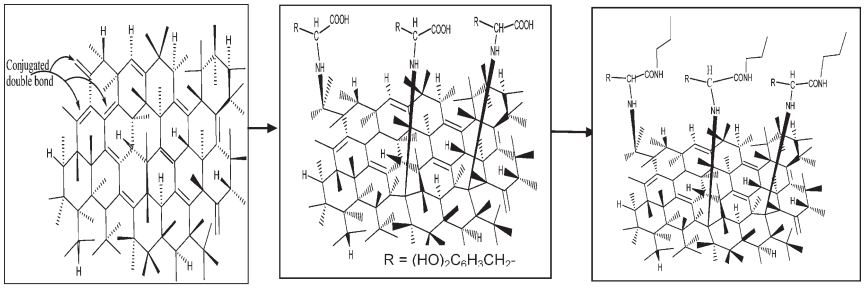


Figure 6. Schematic model of polydopamine coating and the subsequent protein immobilization on HDLC.

immobilized proteins on the BD-HDLC surface (Figure 5d). Since the HDLC surface is a hydrophobic surface, it is possible that the few immobilized proteins on the HDLC surface are due to hydrophobic interactions,[19,20] whereas the large number of immobilized proteins on the BD-HDLC surface is due to covalent interactions. Thus protein immobilization onto the HDLC surface via hydrophobic interaction is weaker than that onto the D-HDLC surface via covalent interaction. Moreover, the AFM image (Figure 5d) is reproducible; there is no removal of proteins due to vertical force between the AFM probe-tip and the protein during noncontact AFM imaging. Thus it could be stated (conjectured or assumed) that the covalent bond-rupture force due to specific chemical interactions between the silicon probe tip of AFM and the BSA protein is negligibly small.

Since BSA protein is a complex molecule having a large variety of functional groups, at this stage it is very difficult to identify the exact route of reaction pathways for D-HDLC with BSA protein. There were two reactive functional groups, namely secondary amine (−NH−) and carboxylic (−COOH) groups in D-HDLC.

As the secondary amine (−NH−) group was more sterically crowded in comparison to free carboxylic acid group (−COOH), there was more possibility for the reaction of carboxylic groups with the BSA protein. Thus the probable scheme for the immobilization of BSA protein onto HDLC via covalent interaction has been described in Figure 6.

(28) Mitsakakis, K.; Lousinian, S.; Logothetidis, S. *Biomol. Eng.* **2007**, *24*, 119–124.

4. Conclusions

We have demonstrated that HDLC films produced by a reactive gas−plasma process,[12] comprised of an ordered hexagonal structure of carbon atoms, can be deposited on large-area-substrate surfaces, having large specific surface area. Large specific surface area is useful for high loading capacity of proteins. The crystalline order of deposited films rivals that of graphene and pyrolytic carbon (HOPG). We further demonstrated that HDLC is an atomically smooth substratum useful for covalent protein immobilization via DOPA as the intermediate layer. Good AFM imaging of protein and retention of the conformation of the immobilized protein are due to the atomically smooth surface with roughness of < 0.05 nm. The ability to coat macroscopic surfaces presents HDLC as a potentially useful coating for biomedical/biosensor devices. Amorphous DLC materials have been shown to exhibit biocompatible surface properties.[21,28] It can be anticipated that HDLC will also be biocompatible and may offer improved properties related to the highly ordered structure.

Acknowledgment. N.R.R. thanks the Department of Atomic Energy, Govt. of India, for funding the XI-plan project *Diamond Nanotechnology for Bioapplications*. The authors also thank Mr. S. S. Sil, Mr. U. S. Sil of PPD, SINP, Mr. A. K. Das of CSD, SINP, and Mr. Anik Dasgupta for their technical help during the experiments in this work, and Mr. Mahendra Babu, RRCAT, Indore, for preparation of samples required for transmission electron microscopy.