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ORIGINAL PAPER

Adsorption of humic acid by amine-modified nanocellulose: an experimental and simulation study

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Abstract The purpose of this study was to evaluate the adsorption of humic acid (HA) by amine-modified nanocellulose. At first, nanocellulose was synthesized by acid hydrolysis, and then was modified by *N*-(2-aminoethyl)-3-aminopropylmethyldimethoxysilane. In the next step, HA and amine-modified nanocellulose were incubated at different conditions (pH, incubation time, temperature, and concentration). Furthermore, we used molecular dynamic (MD) simulation for observation of adsorption at molecular scale. For this purpose, HA and amine-modified nanocellulose were prepared by HyperChem software (Hypercube, Inc., USA), and inserted in Ascalaph Designer 1.8.69 software. Once equilibrium was reached, interactions were simulated for 1,000 ps. Finally, different parameters such as interaction energy, potential energy, and root-mean-square displacement (RMSD) were measured. This study demonstrated that the adsorption of HA was pH-, temperature-, dose-, and time-dependent. Based on simulation results, the interaction energy was reduced, but potential energy and RMSD were increased at the final stage of simulation, which indicated the adsorption of HA. The authors suggest amine-modified nanocellulose for removal of HA in wastewater.

Keywords Humic acid · Adsorption · Amine-modified nanocellulose · Experimental · Simulation · Wastewater

Introduction

Various pollutants may exist in water, which decrease the quality of environmental water, and affect the health of human population and animals (Jonglertjunya and Lertchutimakul 2012). Among the environmental harmful pollutants, humic substances (HSs) are very important, which are formed by decomposition of plant and animal biomass. Regarding the chemical structure, they have a skeleton of aromatic units with different functional groups (e.g., carboxyl, phenolic, hydroxyl, and quinine). HSs are categorized into three compounds based on solubility, including humic acids (HAs), fulvic acids (FAs), and humans (Trump et al. 2006). HSs can cause different environmental and health problems including: (1) undesirable color and taste, (2) binding to heavy metals and biocides which yield new toxic materials, (3) reaction with

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chlorine to form potentially carcinogenic compounds (e.g., trihalomethane), and (4) reduction of adsorption rate and equilibrium capacity of other compounds (Giasuddin et al. 2007). To remove HA, different techniques have been studied including ion exchange, sorption, reverse osmosis, ultra-filtration, flotation, bio-flocculation, electro-coagulation, and chemical oxidation (Bazrafshan et al. 2012).

Moreover, activated carbon (Terdkiatburana et al. 2008), minerals (Davis and Gloor 1981; Liang et al. 2011), montmorillonite (Lin and Juang 2002), zeolite (Wang et al. 2008), and other adsorbents have been used to remove HA from wastewater. It must be mentioned that these adsorbents have different capacities, kinetics, and isotherms. It has been shown that various parameters can affect HA adsorption, such as pH, salt levels, particle size, and molecular weight of HA, initial concentration of HA and adsorbent, incubation time, and temperature (Qin et al. 2012). Since there are various functional groups on the HA, different adsorption mechanisms (e.g., electrostatic, hydrogen band, hydrophobic force, etc.) might be involved in case of HA adsorption.

Although the HA adsorbents have good efficacy, some problems have been observed. For example, activated carbon is not suitable for HA with high molecular weight (Moussavi et al. 2011). On the other hand, zeolite and montmorillonite have heavy metal oxide which may be released during adsorption. Heavy metal oxides (e.g., TiO_2) have cytotoxicity and can release toxic ions. The problems are remarkable, especially for nanosized metal oxides (Jin et al. 2011; Godinez et al. 2013).

The aim of this study was to synthesize a new adsorbent and to evaluate its adsorption capacity at different conditions. We selected nanocellulose as an adsorbent because this material is natural, inexpensive, and non-toxic. To improve its efficacy, nanocellulose was modified by amine groups, in order to interact with carboxyl groups of HA. The work was carried out in the Pajoohesh Lab, Yazd, Iran at November 2013.

Materials and methods

Materials

In order to synthesize nanocellulose, the raw cellulose manufactured by My Baby Company (Iran) was used. *N*-(2-aminoethyl)-3-aminopropylmethyldimethoxysilane (AEADMS) was provided from Sigma-Aldrich Company (St Louis, MO, USA). Other chemicals including nitric acid, sulfuric acid, sodium hydroxide (NaOH), and dimethylsulfoxide (DMSO) were sourced from Merck Company, Germany.

Nanocellulose synthesis

For nanocellulose synthesis, acid hydrolysis method was used according to Loelovich et al. with some modifications (Loelovich 2012). Before hydrolysis, 1 g raw cellulose was treated with 25 mL of 5 M NaOH at 37 °C for 1 h, and then rinsed with distilled water (DW). In the next step, 25 mL of 1 M DMSO was added to NaOH-treated cellulose, and incubated at 37 °C for 1 h, too. Then, DMSO-treated cellulose was washed three times with DW and used for the next step. One gram of NaOH-DMSO-treated cellulose was added to 1 mL of acid mixture (70 % v/v) and incubated at room temperature for 30 min. The acid mixture had sulfuric acid (85 % v/v) and nitric acid (5 % v/v). To complete hydrolysis, some droplets of 90 % v/v sulfuric acid were added, until the milky color of cellulose was removed. Then, 2 mL of 5 M NaOH was gently added to hydrolyzed cellulose, shaken for 5 min, centrifuged at 3,000 rpm for 5 min, and then nanocellulose pellets were washed by DW three times. In the final step, nanocellulose at concentration of 500 µg/mL was prepared in DW, shaken for 5 min, and stored in 5 °C.

Modification of nanocellulose

To modify nanocellulose, 10 mL of nanocellulose at concentration of 500 µg/mL was added to 10 mL of 4 % v/v AEADMS, incubated for 24 h at 37 °C, and centrifuged at 5,000 rpm for 15 min. The pellet (amine-modified nanocellulose) was washed with DW, and its serial concentrations (1,000, 500, 250, 125, and 62 µg/mL) were prepared in DW and stored at 4 °C.

Characterization of amine-modified nanocellulose

The structure, size distribution, and surface composition of amine-modified nanocellulose were investigated by transmission electron microscopy (TEM) (Hitachi, Japan), dynamic light scattering (DLS) (Malvern Instruments, Italy), and Fourier transform infrared spectroscopy (FTIR) (ELICO, India), respectively. For DLS, the viscosity of amine-modified nanocellulose was obtained by DLS apparatus, and then analysis was carried out at 25 °C after stabilization. For FTIR, all samples including nanocellulose, AEADMS, and amine-modified nanocellulose were dried on KBr block, and their adsorptions were read at 500–3,500 cm^{-1}.

The effect of different parameters on the adsorption

To investigate the effect of various conditions on the adsorption, different parameters (e.g., concentration,

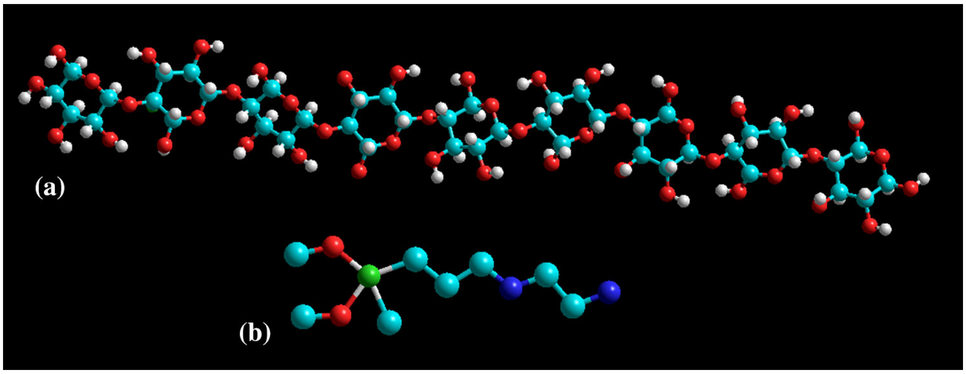


Fig. 1 The structure of nanocellulose (**a**) and AEADMS (**b**) obtained from HyperChem software. In this study, a 9-unit cellulose was considered as nanocellulose

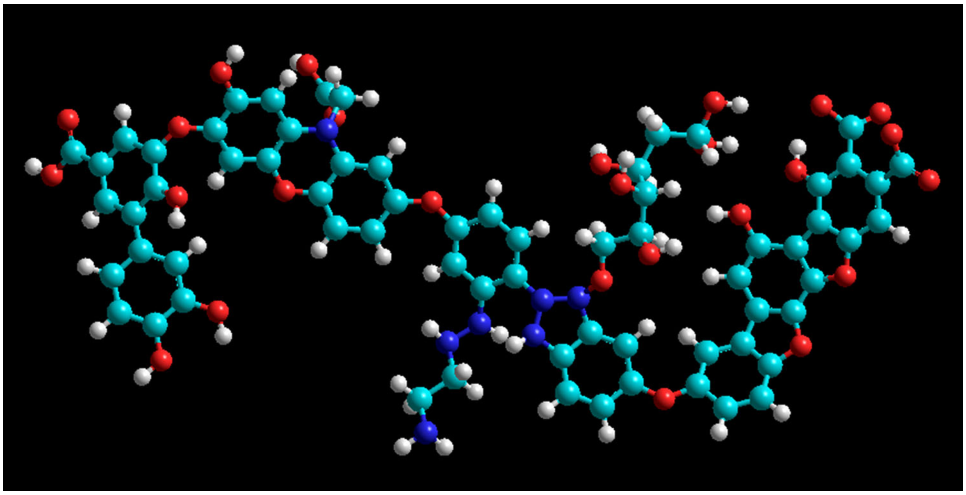


Fig. 2 The structure of humic acid as displayed by HyperChem software

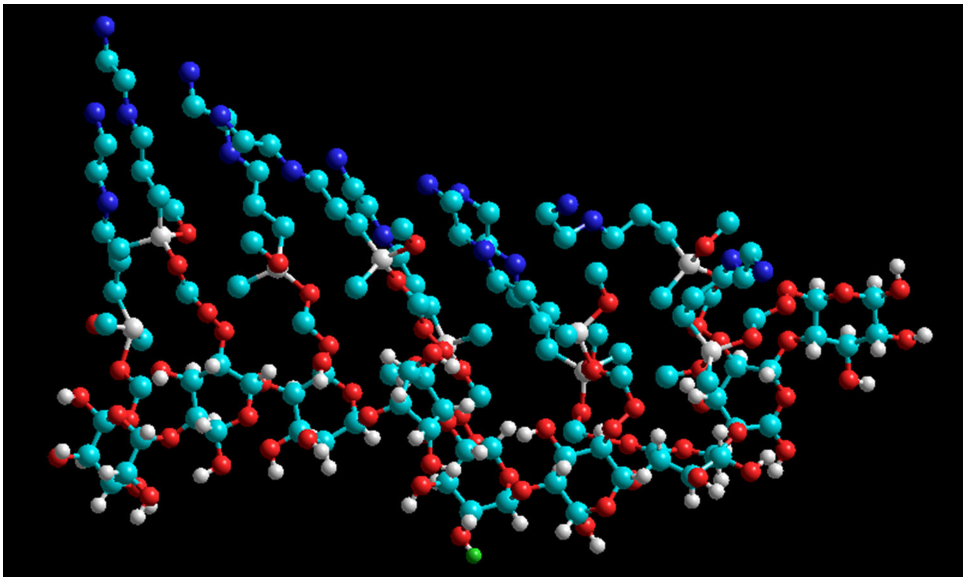
temperature, incubation times, and pH) were studied. In the first group, one mL of serial concentrations of amine-modified nanocellulose was separately added to 1 mL of HA at concentration of 40 µg/mL at pH 7, and separately incubated for 5 min at 4, 25, 37 °C, and 50 °C. In the second group, serial concentrations of amine-modified nanocellulose and 40 µg/mL HA at pH 7 were separately incubated at 25 °C for 5, 10, 15, and 20 min. In the third group, serial concentrations of amine-modified nanocellulose and 40 µg/mL HA at different pH (2, 5, 7, 9, and 12) were separately incubated at 25 °C for 5 min. In the fourth group, serial concentrations of HA (40, 60, 80, 100 µg/mL) were incubated with 1,000 µg/mL amine-modified nanocellulose at optimum pH, incubation time, and temperature which were obtained from three initial experiments. After incubation, all tubes were centrifuged at 10,000 rpm for 5 min, and optical density (OD) of supernatant was read by UV–visible spectrophotometer (ELICO, India) at 254 nm. The adsorption percent (%) was measured by the adsorption equation. As negative control, HA was not incubated with amine-modified nanocellulose.

$$\text{Adsorption }\% = (\text{OD}_{\text{test}} - \text{OD}_{\text{control}}) \times 100/\text{OD}_{\text{control}}$$

Molecular dynamic (MD) simulation

Initial structures of nanocellulose (Fig. 1a), AEADMS (Fig. 1b), HA (Fig. 2), and amine-modified nanocellulose (Fig. 3) were performed by HyperChem Professional 8.0.3 software (Hypercube, Inc., USA) on an Acer laptop with CPU B960. The MD simulations were carried out with the Ascalaph Designer 1.8.69 software on the Inspur Cluster. In this study, force field and topology parameters were assigned from AMBER 99. Also, TIP3P water model with a cubic box (10 Å) was used to solvate the amine-modified nanocellulose and HA. The cutoff distance of electrostatic interaction was 10 Å, and Van der Waals interactions were turned off between 12–14 Å. Firstly, the energy of the whole system was minimized with 1,000 steps with hybrid

Fig. 3 The structure of amine-modified nanocellulose after geometry optimization, prepared by HyperChem software



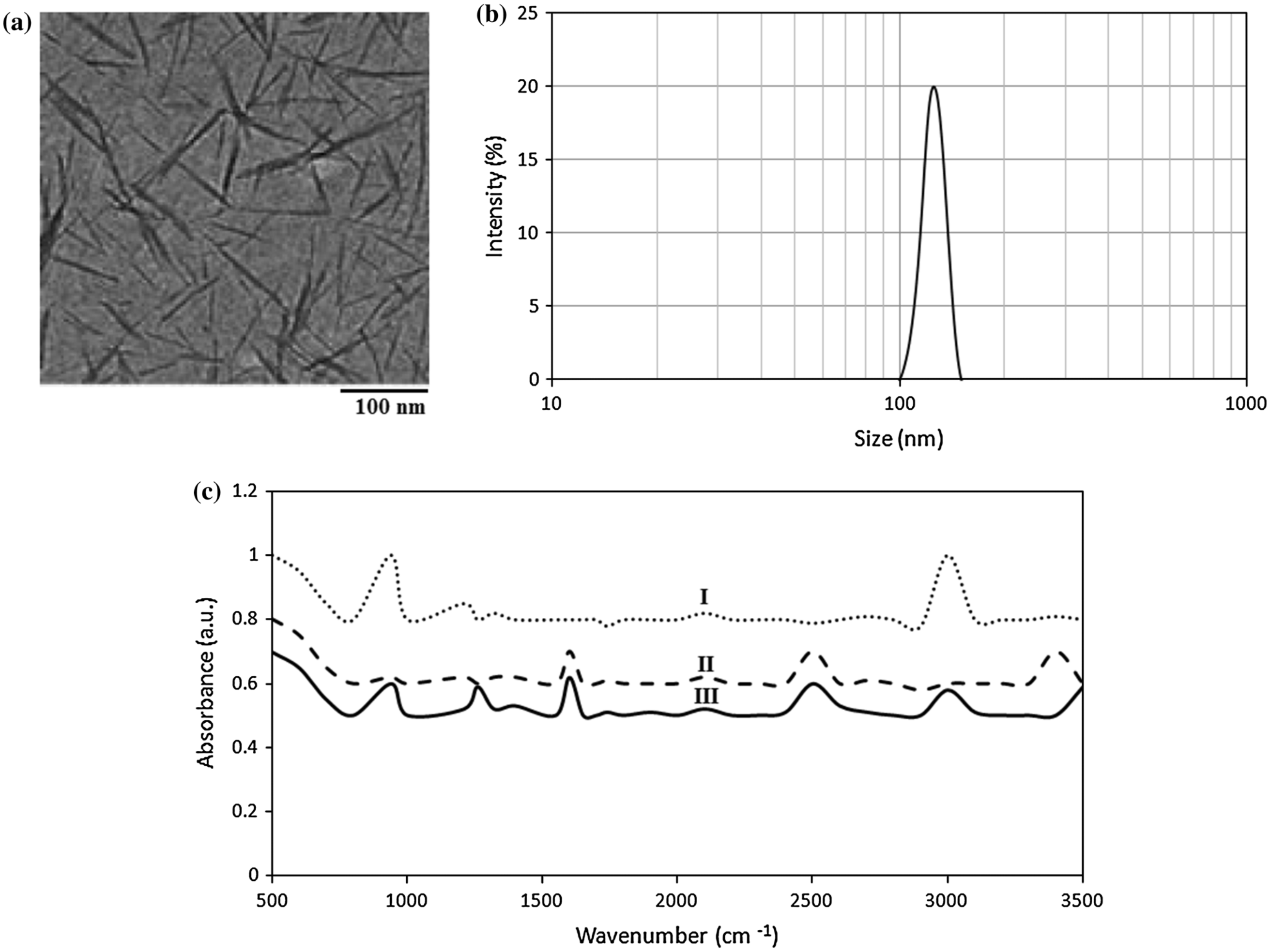
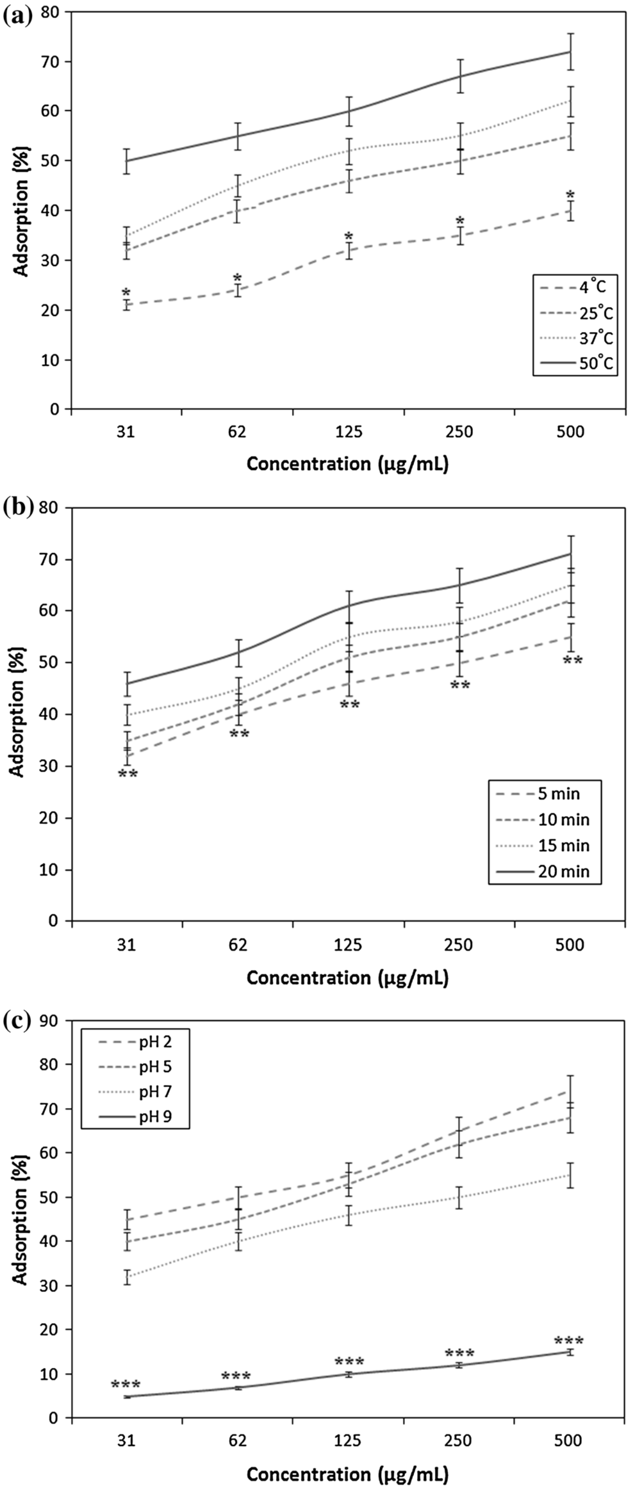


Fig. 4 The characterization results. The TEM image (**a**) and DLS graph (**b**) of amine-modified nanocellulose, also with the FTIR spectrum (**c**) of nanocellulose (I), AEADMS (II), and amine-modified nanocellulose (III)



◀**Fig. 5** The effects of temperature (**a**), incubation time (**b**), and pH (**c**) on the humic acid adsorption. In the (**a**) section, temperature was variable and the other parameters were constant (pH = 7, incubation time = 5 min). In the (**b**) section, incubation time was variable and the other parameters were fixed (pH = 7, temperature = 25 °C). In the (**c**) section, pH was variable and other two parameters were constant (incubation time = 5, temperature = 25 °C). $*P < 0.05$ when comparing the adsorption at 4, 25, 37 °C for the same concentration. $**P < 0.05$ when comparing the adsorption at 15 min and 20 min for the same concentration. $***P < 0.05$ when comparing the adsorption at pH 2, 5, and 7 for the same concentration

Lui Storey and Conjugate Descent (gradient = 0.001 and iteration = 100), and then MD simulation was carried out for 1,000 ps in NVT ensemble at 298 K. At the end of simulation, different parameters such as interaction energy, potential energy, and root-mean-square displacement (RMSD) were obtained from simulation software. Also, the ultimate structure of HA and amine-modified nanocellulose were captured at the final step of simulation.

Statistical analysis

The results are shown as the mean ± standard deviation (SD) with three independent tests. Parametric test (Student's *t* test) was applied to evaluate the significant difference between groups by means of SPSS software (v.16.0 for Windows; SPSS Inc., USA). $P < 0.05$ was considered as significant difference.

Results and discussion

Characterization

The TEM image and DLS graph of amine-modified nanocellulose are shown in Fig. 4a, b, respectively. Based on TEM image, amine-modified nanocellulose was approximately rod-like (100 nm × 5 nm). DLS graph showed that the size distribution of amine-modified nanocellulose was near 100–150 nm. The FTIR spectrum of nanocellulose (I), AEADMS (II), and amine-modified nanocellulose (III) are shown in Fig. 4c. As is seen, nanocellulose had sharp peaks at 940 and 3,000 cm^{-1}, but AEADMS showed sharp peaks at 1,600, 2,500, and 3,400 cm^{-1}. As is observed, both specific peaks of nanocellulose and AEADMS were observed in the spectrum of amine-modified nanocellulose. Generally, the spectrum of amine-modified nanocellulose shows the attachment of AEADMS to the nanocellulose.

The effect of various conditions on the adsorption

The effects of temperature, incubation time, and pH on the adsorption are shown in Fig. 5a, b, c, respectively. As is shown, when temperature and incubation time were increased, the adsorption was also raised. In contrast, the adsorption was decreased with increase in pH. In all experiments, the highest and the least adsorption were shown at amine-modified nanocellulose concentration of

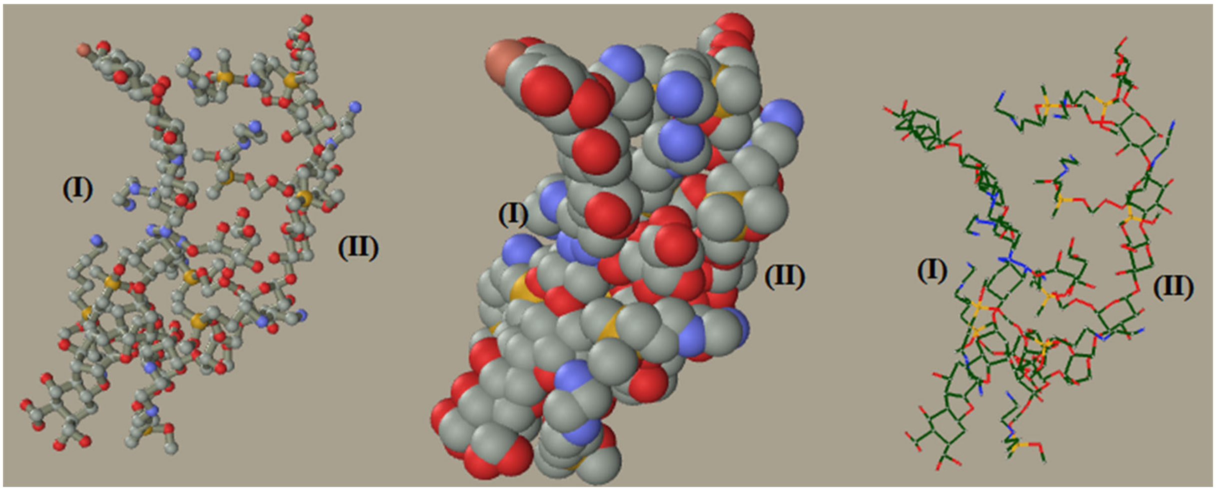


Fig. 6 The 3-dimensional structure of humic acid (I) and amine-modified nanocellulose (II) at the final simulation by the HyperChem software. These structures are shown as ball and stick (*left*), Corey-Pauling-Koltun (CPK) (*middle*) and CPK wire frame (*right*)

500 µg/mL and 31 µg/mL, respectively. This study showed that HA adsorption was dose-dependent. As is seen, significant differences were found between HA adsorption at 4 °C versus 25, 37, and 50 °C ($P < 0.05$). Such difference was also seen between HA adsorption after 5 versus 10, 15, and 20 min ($P < 0.05$). Likewise, there were significant differences between HA adsorption at pH 9 versus pH 7, pH 5, and pH 2 ($P < 0.05$). Details of statistical analysis are demonstrated on the figures. This study showed that the maximum adsorption was approximately 98 %, with optimum conditions, temperature = 50 °C, pH = 2, incubation time = 20 min, concentration of amine-modified nanocellulose = 500 µg/mL, and concentration of HA = 40 µg/mL.

According to results, the adsorption was increased when incubation time and temperature were raised, which may be due to higher interaction between HA and amine-modified nanocellulose. In contrast, the adsorption was decreased with increase in pH. It may be that amine groups of amine-modified nanocellulose and carboxyl groups of HA are differently affected by basic and acidic conditions. This phenomenon changes electrostatic forces and leads to different adsorption at different pH. There is no published paper to show the application of amine-modified nanocellulose for absorption of HA, and the present study is the first report.

In this section, we will discuss on other amine-modified adsorbents which have been investigated in previous studies. Zhang et al. studied mechanisms and kinetics of HA adsorption onto chitosan-coated granules. They indicated that the adsorption was strongly pH-dependent, i.e., high amounts of HA were adsorbed at neutral and acidic conditions but the adsorption was reduced remarkably with increase in pH. According to X-ray photoelectron spectroscopy experiment, the amine groups of chitosan were protonated after HA adsorption and led to organic complex formation between the protonated amino groups and HA (Zhang and Bai 2003). Anirudhan et al. worked on surfactant-modified bentonite (SMB) for the removal of HA from wastewaters. They showed that SMB had very high (99 %) adsorption rate at acidic condition. It was indicated that the maximum adsorption capacity was 73.52 µmol/g at pH 3.0. Also, the adsorption of HA was less with increase of ionic strength due to the formation of surface complexes on SMB. They declared that SMB efficacy was not changed after three times of usage (Anirudhan and Ramachandran 2007). In another study, Anirudhan et al. studied on amine–modified polyacrylamide–bentonite composite for the adsorption of HA. In the study, amine modification was attained by direct-intercalation polymerization. They found that the maximum adsorption was 99.9 % at pH range of 4.0–7.0. It was explained that the mechanism of adsorption was mainly through the complex formation between the protonated NH2 groups in amine–modified polyacrylamide–bentonite and the carboxyl groups in HA. A pseudo-second-order model was used to describe the kinetics of adsorption, and the adsorption was increased in higher temperatures, indicating endothermic nature of adsorption (Anirudhan et al. 2008). Zhan et al. used bilayer hexadecyltrimethyl ammonium bromide-modified zeolite (BHABMZ) to remove HA from aqueous solution. Their results showed that BHABMZ had higher adsorption rate than the natural zeolite. They indicated that the coexistence of Ca^{2+} in solution favored adsorption onto BHABMZ. On the other hand, adsorption capacity was decreased with increasing pH. They indicated that the adsorption of HA was well fitted with pseudo-second-order kinetic model and the Langmuir model (Zhan et al. 2011).

Tanga et al. declared that amine-functionalized magnetic mesoporous composite microspheres had high

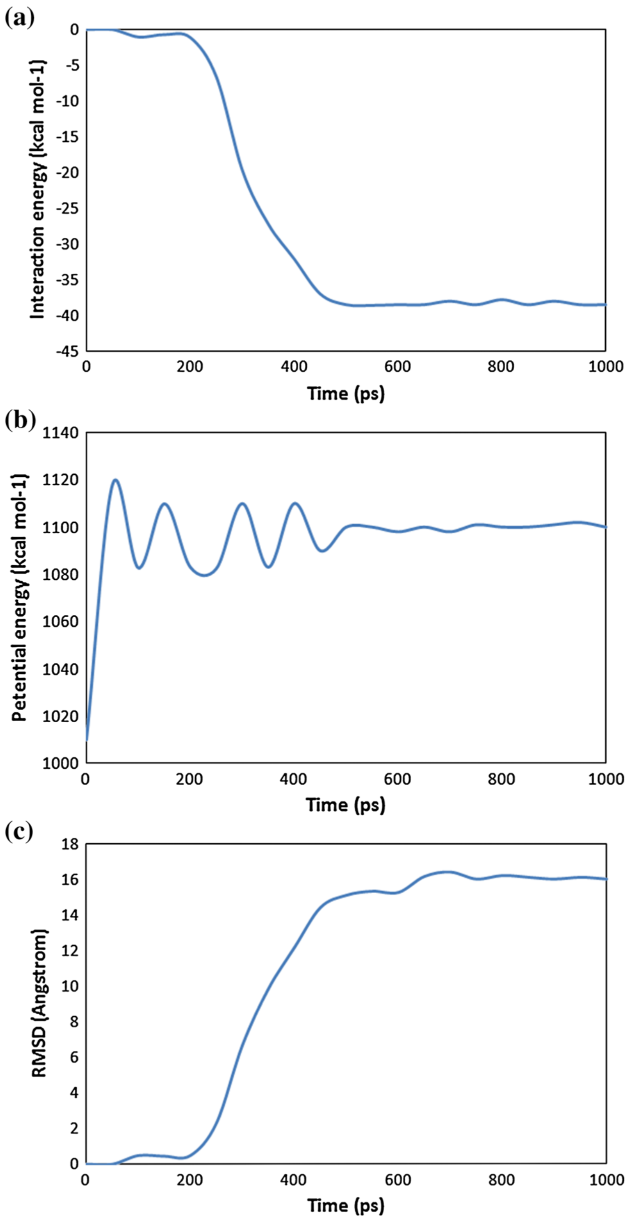


Fig. 7 The molecular dynamics simulation results: Interaction energy (**a**), potential energy (**b**) and RMSD (**c**). RMSD: root-mean-square displacement

adsorption affinity to HA with a maximum adsorption capacity. The Freundlich model and the pseudo-second-order kinetic were well describing the adsorption. These studies indicated that amine group could easily interact with carboxyl groups of HA, and the fact leads to HA adsorption (Tanga et al. 2012). In consistent with these studies, we showed that amine-modified nanocellulose is a good HA adsorbent.

Regarding the application of nanoparticles for removing HA, Qiao et al. showed that nano-photocatalyst Fe_2O_3/TiO_2 could remove HA. They explained that degradation and decolorization of HA were obtained with higher efficiency in acidic condition. First-order reaction kinetic was suitable for photocatalytic degradation (Qiao et al. 2003). Yang et al. worked on the adsorption behavior of HA by TiO_2, SiO_2, Al_2O_3, and ZnO nanoparticles. It was declared that TiO_2, Al_2O_3, and ZnO nanoparticles could absorb HA but SiO_2 nanoparticle had no efficacy. They discussed that HA adsorption was mainly induced by electrostatic forces and ligand exchange between HA and nanoparticle surfaces (Yang et al. 2009). According to previous studies, different kinetic models were introduced to predict the mechanism of adsorption [including pseudo-first-order model, pseudo-second-order model, first-order reversible reaction model, Weber and Morris sorption kinetic model, external mass transfer model, Elovich's model, first-order equation of Bhattacharya and Venkobachar, and Ritchies's equation (Febrianto et al. 2009)].

MD simulation

Figure 6(I) and (II) show the structure of amine-modified nanocellulose and HA at the final simulation, respectively. These figures demonstrate that HA was absorbed by amine-modified nanocellulose. The interaction energy, potential energy, and RMSD are shown in Fig. 7a, b, and c, respectively. As is seen, interaction energy was reduced and equilibrated at −40 kcal/mol, but potential energy was increased and balanced at near 1,100 kcal/mol. Similar to potential energy, RMSD was increased and equilibrated at near 16 angstrom. Generally, these findings confirm the adsorption of HA by amine-modified nanocellulose.

In this study, MD simulation was also used to show interaction of HA and amine-modified nanocellulose molecules. All simulation results (e.g., interaction energy, potential energy, and RMSD) approved the adsorption of HA by amine-modified nanocellulose, too. Regarding its molecular structure, HA was fitted with amine-modified nanocellulose. Based on simulation data, the authors hypothesize that carboxyl and hydroxyl groups of HA interact with amine group of amine-modified nanocellulose, which may lead to a new compound. We propose that electrostatic forces are the main mechanism of adsorption. There is no computer-based study to compare results, and this study is the first report. Shih et al. showed the adsorption kinetics and thermodynamic properties of HA by toluene. They indicated that MD simulation is an alternative and powerful tool to understand the adsorption at the molecular level (Shih et al. 2006). Taken together, the adsorption of HA by amine-modified nanocellulose was depended on pH, temperature, incubation time, HA concentration, and amine-modified nanocellulose concentration. On the other hand, MD simulation showed the adsorption. The authors suggest that amine-modified nanocellulose must be studied in actual conditions to remove HA from wastewater.

Conclusion

This study showed that the adsorption of HA by amine-modified nanocellulose was pH-, temperature-, dose-, and time-dependent. Furthermore, MD simulation confirmed HA adsorption. The authors propose that amine-modified nanocellulose may be used for removal of HA from wastewater.

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Conflict of interest No conflict of interest is addressed.

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