Synthesis and characterization of Dopamine graft compound N-methacryloyl 3,4-dihydroxyl-phenylamine

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Abstract. In order to obtain adhesive biomaterials inspired by mussels, the intermediate derivatives of dopamine, N-methacryloyl 3,4-dihydroxyl-phenylamine (dopamine methacrylamide DMA), was synthesized by grafting methacrylate anhydride to dopamine. The structure of the compound was confirmed by fourier transform infrared spectroscopy and nuclear magnetic resonance. The thermal stability of DMA was also characterized by thermo gravimetric analysis and differential scanning calorimeters techniques. The surface morphology of DMA crystal was analysed by scanning electron microscope analyses. The present result showed that the synthesis of new monomers was successfully fulfilled and the new compounds retain the hydroxyl functional groups. The surface morphologies and thermal stability of DMA crystal were also altered by grafting reaction.

1. Introduction

Adhesive biomaterials have many potential medical applications, such as wet tissue adhesives in minimally invasive surgery, and as vehicle for drug delivery to mucosal surfaces. Clinically successful adhesive biomaterials should have the following primary attributes: (1) the ability to rapidly solidify or polymerize in situ from a fluid precursor; (2) the ability to form strong and durable bonds to tissue surfaces even in the biological environment; and (3) biocompatibility. However, the current medical adhesives seldom meets these requirements due to lack of adhesion strength, in particular, in the presence of biological fluids, sensitization and allergic response, and inflammation. Therefore, it is necessary to develop the safe and effectively curable adhesive biomaterials on wet tissue surface with minimal inflammatory insult.

Marine and freshwater mussels are attracted many attentions due to their remarkable adhesive capabilities to natural or manmade surfaces in the wet environment. Mussels secrete specialized adhesive proteins containing a high content of L-3,4-dihydroxyphenylalanine(DOPA), which residue is thought to be responsible for adhesion. Proteins incorporating the DOPA functionality contribute to strong adhesive structures, allowing marine organisms to fix their bodies to various types of surfaces, such as polymers, ceramics, and metals, even in aqueous conditions. Under oxidizing conditions, the covalently cross-linking occurs between DOPA molecules or between DOPA molecule and biologically relevant nucleophiles, such as primary amines and thiols, by reversal of the Michael addition. Lee et. al. demonstrated that the adhesive strength of DOPA was declined compared to that of the unoxidized DOPA, in particular when it adhered to metallic surface, by the atomic force micro-
The oxidized forms of DOPA that result from such reactions are believed to be less adhesive than unoxidized DOPA. Therefore, several groups have reported the synthesis and characterization of DOPA-containing peptides and polymers to avoid the oxidization of DOPA and to maintain its adhesive. However, most previous efforts incorporating DOPA into polymer have been hypothesized to reduce the adhesive properties of DOPA and require reagents that are harmful to biological tissues. Both adhesive and polymerize in situ of DOPA are depended on the two hydroxyl in the benzene. DOPA containing proteins and polymers exhibit better adhesion to both metallic and mucosal surfaces when DOPA is not oxidized. Hence it is necessary that two hydroxyl on the DOPA are preserved when the solidification of the liquid adhesive to improve adhesive strength.

The aim of this research was to graft methacrylate anhydride with DOPA in order to develop materials for the adhesive biomaterials. Monomers of N-methacryloyl 3,4-dihydroxy phenylamine was synthesized and these monomers were confirmed by fourier transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR) techniques. The thermal stability of DMA monomer was characterized by thermo gravimetric analysis (TG) and differential scanning calorimetry (DSC). The morphologies of DMA crystal were observed by Scanning Electron Microscopy (SEM).

2. Experimental

2.1. Materials

3,4-dihydroxyphenethylamine hydrochloride (dopamine-HCl) were purchased from Sigma (Stiernem, Germany); methacrylate anhydride were purchased from Aladdin (Shang Hai, China); sodium borate, sodium bicarbonate, sodium hydroxide, hydrochloric acid, tetrahydrofuran, ethylacetate and hexane were purchased from Kelong (Cheng Du, China).

2.2. Synthesis of DMA

20g of \( \text{Na}_2\text{B}_4\text{O}_7 \) and 8g of \( \text{NaHCO}_3 \) were dissolved in 200mL of deionized water and bubbled with \( \text{N}_2 \) for 20 min. 10g of dopamine-HCl (52.8 mmol) was then added followed by the dropwise addition of 9.4 mL of methacrylate anhydride (58.1 mmol) in 50 mL of tetrahydrofuran, during which the pH of solution was kept above 8 with addition of 1mol/L \( \text{NaOH} \) as necessary. The reaction mixture was stirred 12h at room temperature with \( \text{N}_2 \) bubbling. At this time, a white slurry-like solution had formed and was then washed twice with 50 mL of ethyl acetate. The resulting solid in the solution was vacuum filtered and the obtained aqueous solution was acidified to pH 2 with 6M of HCl solution. The organic layer of the solution was extracted three times from the acidified aqueous solution with 50 mL of ethyl acetate. The extracted clear brown organic layer in the ethyl acetate was dried over MgSO\(_4\). The solution volume was reduced to 25 mL with a rotary evaporator. The obtained solution was added to 250 mL of hexane with vigorous stirring to precipitate a brownish solid and then the formed suspension was refrigerated to maximize crystal formation size. To purify, the resulting light brown solid was dissolved in 20 mL of ethyl acetate and precipitated in 300 mL of hexane. The final solid powder was dried in a vacuum overnight. The synthetic route of the DMA monomer and structure of the product were shown in Figure 1 and Figure 2.

![Figure 1. Synthetic route of DMA](image1)

![Figure 2. Structure of DMA](image2)
2.3. FT-IR spectroscopy

FT-IR spectroscopy (5700, Instron, USA) was performed to analysis DMA composition. Small amount of DMA was ground together with KBr and then pressed into pellets (2mg/300mg KBr) for FT-IR with a resolution of 4.00 cm$^{-1}$. Infrared spectra were recorded in the range 4000-400 cm$^{-1}$ to evaluate the molecular structure.

2.4. Nuclear magnetic resonance (NMR) spectroscopy

$^1$H NMR and $^{13}$C NMR spectra of DMA monomers were investigated on (AC-E200 Bruker) monomers with CD$_3$OD as the solvent and chemical shifts (δ) are given relative to tetramethylsilane as the internal standard.

2.5. SEM analyses

Surface morphology of DMA monomers was observed by SEM. The samples were coated with a thin layer of Gold (Au) by sputtering (450X, Emitechk, England) and then the morphology of them were observed on a scanning electron microscope (JSM-7001F, Jeol, Japan) that operated at the acceleration voltage of 15 kV.

2.6. DSC analysis

The DSC spectra of DMA was obtained on Perkin-Elmer DSC Model 7. Measurements were performed over the temperature range of 25-500°C at the heating rate of 5°C/min in hermetically sealed aluminium pans.

2.7. TGA analysis

Thermal stability of the DMA was examined, from 25 to 500°C heated at 5°C/min in nitrogen gas flushed at 200ml/min. The samples were subjected to thermo gravimetric analysis (TGA) to determine the decomposition temperatures.

3. Results and discussion

3.1. FT-IR spectra

Figure 3 shows the FTIR spectrum of the DMA. The characterization adsorption peaks at 3070cm$^{-1}$ and 3170~ 3350cm$^{-1}$ (were assigned to the Chung amide N-H stretching). The characterization adsorption peak at 1659, 1550 and 1260cm$^{-1}$ were owing to acuate peak amide I C=O stretching, double pe-
akamide II, and C–N stretching for amide III). This is an evidence of the grafting from N atom positions of the monomer.

3.2. NMR spectra

The $^{13}$C NMR and $^1$H NMR spectra of DMA were showed in Figure 4 and Figure 5. A total of 12 carbon signals were found in the spectra (Figure 2). $^{13}$C NMR: δ171.2 (C9), δ146.1 (C10), δ144.3-116.3 (C1-C6), δ132.0 (C-11), δ42.6 (C-8), δ35.7 (C-7), δ18.7 (C-12).

![Figure 4. $^{13}$C NMR of DMA](image)

$^1$H NMR δ: 1.89 (3H, s, C(=O)-C(-C$\text{H}_3$)=CH$_2$), 2.65 (t, 2H, C$\text{H}_3$OH$\text{H}_3$-C$\text{H}_2$-CH$_2$(NH)-C(=O)-), 3.37 (m, 2H, C$\text{H}_3$(OH)$\text{H}_2$-C$\text{H}_2$(NH)-C(=O)-), 5.31 (s, 1H, C(=O)-C(-CH$_3$)=CHH), 5.63 (s, 1H, C(=O)-C(-CH$_3$)=CHH), 6.52 (d, 1H, C$\text{H}_3$H(OH)$_2$-), 6.66 (m, 2H, C$\text{H}_3$H(OH)$_2$-).

![Figure 5. $^1$H NMR of DMA](image)
3.3. SEM micrographs of DMA

The morphologies of DMA are illustrated in Figure 6 and Figure 7. The particles of DMA have irregular shapes. The particle sizes of DMA were ranged approximately 10 µm or more. A few big plate-like crystals due to methacrylate anhydride are clearly distinguishable from the DMA particles [15], which display an average size of 1-2 µm. SEM analyses showed the surface morphologies and sizes of DMA were altered by grafting reaction.

![Figure 6. SEM micrograph of the DMA](image1)

![Figure 7. SEM micrograph of the DMA](image2)

3.4. Thermal analysis

The DSC and TG curve of DMA are shown in Figure 8. The obtained DSC curve showed the marked endothermal peaks at 120-160 °C. The maximum of the endothermal peaks for DMA was at 143 °C, which was its melting point of it. The DSC curves of DOPA did not show a marked endothermal peak like DMA in any temperature range. [16] In general, the DMA powder showed a two-step weight loss as confirmed by DSC; one with a steeper change in the weight loss curve in the temperature range from 248°C to about 343°C, corresponding to the part of C-N bond and thermal decomposition of methacrylate anhydride. Subsequently, a comparatively slow weight loss appeared about 343-400°C due mostly to the elimination of the main component. The melting point, DSC and TG curves of DMA were different from those of DOPA, which was due to grafting of methacryloyl on DOPA.

![Figure 8. DSC and TG curves for DMA](image3)
4. Discussion

L-3,4-Dihydroxyphenylalanine (DOPA) is an unusual amino acid found in mussel adhesive proteins, which is believed to lend adhesive characteristics to these proteins [17]. The hydroxyl on DOPA is believed to be the function group for high adhesives of mussel secretions. Unfortunately, most previous efforts incorporating DOPA into polymer have been hypothesized to reduce the adhesive properties of DOPA due to the hydroxyl group of the catechol structure was easy to be oxidized. In this study, methacrylate anhydride was grafted on DOPA molecule to avoid its oxidization.

It was found that carbon-oxygen double bond absorption peaks appeared in FT-IR and NMR spectra of DMA, which indicated that the new monomer, N-methacryloyl 3,4-dihydroxyl-L-phenylamine, was synthesized successfully. It was also found that peaks of hydroxyl from the FT-IR and NMR spectra, which inferred that the new monomer had the hydroxyl functional groups. The structure of the DMA, which had catechol structure, endowed its potential application as the medical adhesives. DMA was brown powder (ethyl acetate-hexane), insoluble in water, ethanol and ethyl acetate, slightly soluble in chloroform, whereas it was dissolved in DMSO and carbinol. The melting point of DMA is 143°C.

From the SEM micrographs of DMA, it was revealed that morphologies and sizes of DMA particles were altered by grafting reaction. This morphology change probably was the reason for the change of dopamine graft polymer adhesion. The obtained DSC curve of DMA show a marked endothermal peak in the temperature range 120-160°C whereas DSC curve of DOPA did not show any endothermal peak like DMA in any temperature range. It was concluded that due to the grafting of methacrylic anhydride DMA had better crystallinity than DOPA.

5. Conclusions

It is feasible to synthesized N-methacryloyl 3,4-dihydroxyl-L-phenylamine, which still had catechol structure and hydroxyl functional groups, which might be endowed DMA the high adhesion. The present results confirmed the difference between DOPA and DMA in surface morphologies, melting point, DSC and TG spectra.

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