CHAPTER II

MOLECULAR IMPRINTING OF BISPHENOL A-BASED POLYBENZOXAZINES VIA MIXING CURING PROCESS

Abstract

The molecular recognition in polybenzoxazines via molecular imprinting approach prepared by mixing and curing of benzoxazine monomers and template molecule is proposed. Cholic acid (T_d = 370°C) is a successful model template for bisphenol A-based polybenzoxazines under the co-curing conditions of 190°C for 8 h *in vacuo*. The imprinting polybenzoxazine is prepared by removing cholic acid using a solvent extraction method. The curing conditions, the stability, and the chemical structure of the imprinting polybenzoxazines are studied by TGA, DSC, and FTIR. The effectiveness of the imprinting polybenzoxazines to entrap a series of template-like molecules, which are cholic acid, deoxycholic acid, benzoic acid, chloramphenicol, carbaryl, and pyridine, are studied by immersing in each solution and analyzing by UV-vis spectrophotometry.

Keywords: Molecular imprinting, Bisphenol A-based benzoxazines, Cholic acid, Template

Introduction

Molecular imprinting is a method for preparing a synthetic polymer to exhibit the molecular recognition phenomenon.[1-4] In this technique, monomers form a preassembled complex with template molecule via covalent[5-6] or non-covalent bonds.[7] A preassembled complex and monomers or crosslinkers are polymerized to form an imprinting structure with templates.[1-4] After removing template molecules, the polymer network, so-called molecular imprinting polymer (MIP), provides the specific cavity and specific binding site for the template or template-like molecule. The applications of MIP for advanced materials include a stationary phase for chiral separation[8], artificial receptors in drug assays[9-10], and recognition elements in membranes.[11-12]

Polybenzoxazine[13] is a novel phenolic resin with superb physical and mechanical properties. Chirachanchai et al.[14] reported that polybenzoxazine and their related compounds show a unique property to perform as host compounds by controlling the structure at the molecular level. The structure of polybenzoxazines resembles that of calixarenes, consisting of phenolic group and aza-methylene linkages (Scheme I). Thus, the development of benzoxazines for advanced applications[14-15] can be achieved by various molecular designs and chemical modifications.

Previously, Kirsch et al.[16] reported the MIP of divinylbenzene (DVB) to provide the binding sites of phenolic group interacting with heterocycles such as pyridine, quinoline, and acridine. It is important to note that the ring opening polymerization of benzoxazine monomers may imprint template molecules via hydrogen bonding between phenol groups as seen in the case of DVB.

This article is focused on bisphenol A-based benzoxazine monomer for MIP. The MIP is prepared by co-curing of a benzoxazine monomer and template. Hence, the curing temperature is one of the main factors to consider the types of the template molecule. A series of molecules with different functional groups, such as carboxylic acid, carbamates, and heterocyclic compound are studied as template and template-like molecules. The imprinting property of polybenzoxazines is qualitatively and quantitatively analyzed by TGA, DSC, FTIR, and UV-vis spectrophotometry. The

clarification of the imprinting phenomenon will be a guideline for the practical application of polybenzoxazines.

Experimental Section

Materials. Bisphenol A was purchased from Aldrich Chemical Company, Inc. Anhydrous sodium sulfate, benzoic acid, and chloramphenicol were purchased from Fluka Chemicals (Buchs, Switzerland). Isopropanol and pyridine were the products of Lab-scan (Thailand). Methanol was purchased from J.T. Baker (U.S.A.). Aniline and sodium hydroxide were obtained from Ajax Chemicals (Australia). Paraformaldehyde was purchased from E. Merck (Germany). Cholic acid was purchased from TCI group (Japan). Deoxycholic acid was purchased from Nacalai Tesque (Japan). A commercial grade of 1-naphthyl methylcarbamate or carbaryl was received from AG-GRO (Thailand) Co., Ltd. and recrystallized in acetone before use. The other chemicals were used as received.

Procedures. Fourier transform infrared spectra were measured at a resolution of 4 cm^{-1} by using a Bruker Equinox55/S spectrophotometer equipped with deuterated triglycine (DTGS) detector under the constant purge with dry air. Differential scanning calorimetry (DSC) and Thermal Gravimetric Analysis (TGA) were performed by a Perkin-Elmer DSC7 and a DuPont Thermal Gravimetric Analyzer, respectively, using N_2 as a purge gas. For DSC, the sample (10 mg) was sealed in a closed aluminum sample pan and heated from 30°C to 350°C at a heating rate of 10°C/min. For TGA, thermal degradation experiments were done under a rate of 10°C/min from 30°C to 750°C. The template binding ability was qualitatively and quantitatively analyzed by a Perkin-Elmer Lamda-10 ultraviolet-visible spectrophotometer (UV-vis).

Preparation of Bis(3,4-dihydro-2H-3-phenyl-1,3-benzoxazinyl)iso-propane, 1. Compound **1** was prepared according to Ning et al.[13]

Preparation of Molecular Imprinting Polybenzoxazine. Compound **1** (0.5380 g, 1.16 mmol) was mixed with cholic acid (0.4758 g, 1.16 mmol) and heated in vacuum oven at 190°C (0.01 torr) for 8 h. The product was ground and refluxed in

methanol for 12-15 h to completely remove cholic acid. The product was dried *in vacuo* at 60°C for 6 h.

Template Rebinding Procedures. The molecular imprinting polybenzoxazine (50 mg) was immersed in 10 mL of cholic acid solution (10 mL, 10 mM). The mixture was shaken for 15 min and kept in room temperature for 24 h. The polymer was filtered and the concentration of template remaining was measured by UV-Vis. Other template molecules were studied for rebinding with the similar procedures.

Results and Discussion

Optimal Curing Conditions. In order to achieve a successful co-curing with template, the optimal curing condition of bisphenol A-based benzoxazine monomer was studied. The condition obtained would be a guideline to select an appropriate template to form polybenzoxazine framework.

After heating **1** in various temperatures, the curing induced the ring opening reaction resulting in a clear and yellow rigid sheet. When the oxazine ring is opened, hydroxyl groups and tetrasubstituted benzene are consequently generated to be polybenzoxazines (Scheme I). Figure 1 shows the products with a broad peak at 3200 cm^{-1} for hydroxyl group and a peak at 1481 cm^{-1} for tetrasubstituted benzene functional group. The curing temperature at 190°C gives the product with those peaks be more significant peaks as comparing to other curing temperatures.

DSC is applied to confirm the curing of **1**. Figure 2 clarifies that the exothermic peak at 230°C still appears in the cases of the curing products from 130°, 150°, and 170°C. However, for the product obtained from 190°C, the exothermic peak was not observed. This indicates the optimum curing condition of benzoxazine monomer.

Template Selection and Polybenzoxazines Framework Formation. A series of templates, which are cholic acid, carbaryl, and chloramphenicol (Scheme II), having different functional groups to interact with hydroxyl group of polybenzoxazine framework, were selected. It is important to clarify the thermal

stability of the templates under the curing condition required for **1**. Figure 3 summarizes the thermal stability of the templates selected in the present work.

Although the templates have the thermal stability up to 200°C, the curing condition done *in vacuo* at 190°C may induce the degradation in structure. Compound **1** and templates were mixed and co-cured under the optimal condition of 190°C for 8 h *in vacuo*. Figures 4 and 5 show the FTIR spectra of **1**, chloramphenicol, cholic acid, and the product obtained from the curing process. In the case of chloramphenicol, after treating in similar condition as that of optimal curing, the FTIR spectrum (Figure 4(b)) indicates that the compound obtained was already degraded. It was also found that there was no characteristic peak of chloramphenicol appeared after co-curing. Similarly, carbaryl was found to be degraded from this curing condition. Hence, it was concluded that chloramphenicol and carbaryl were not appropriate templates.

In contrast, cholic acid shows that the structure maintained even after treating in the curing condition (Figure 5(a), 5(b)). Figure 5(c) shows that the co-curing product of cholic acid and bisphenol A-based benzoxazine monomer. The spectrum shows a broad band at 3400 cm^{-1} due to the OH group of the open ring structure of the polybenzoxazine and a new peak at 1730 cm^{-1} belonging to the carbonyl group of the cholic acid. The free OH peak at 3525 cm^{-1} which appeared in cholic acid is changed to a broad peak at 3388 cm^{-1}, suggesting the formation of hydrogen bonding of cholic acid with the framework of polybenzoxazines. This indicates that compound **1** was polymerized incorporating cholic acid as a template molecule. The stability of cholic acid in the polybenzoxazine framework was clarified by DSC. As shown in Figure 6, polybenzoxazines with cholic acid gives the endothermic peak at 200°C, which corresponds to the melting point of cholic acid, while there is no exothermic peak observed. This indicates that cholic acid is maintained in the framework while the curing is accomplished.

Template Removal. Initially, template removal was preliminary studied by immersing the cholic acid-polybenzoxazine co-cured product in MeOH for overnight. It was found by FTIR that the removal was not successful. This might be due to the strong hydrogen bonded network in the polymer. An attempt to remove

the cholic acid as well as the unreacted benzoxazine monomer to obtain the purified imprinting polybenzoxazine was done by refluxing in MeOH overnight. Figure 7 shows the FTIR spectra of products before and after the extraction. The cholic acid was removed completely from the polybenzoxazine framework as confirmed from the disappearance of the carbonyl peak at 1730 cm^{-1}. In addition, the structure of the framework was still maintained after template removal as suggested by the tetrasubstituted benzene peak of polybenzoxazine at 1481 cm^{-1} (Figure 7).

Figure 8 shows DSC thermograms of polybenzoxazines with cholic acid before and after the extraction. The melting peak belonging to cholic acid (200°C) disappeared after the product was refluxed with MeOH. This implies that cholic acid is no longer a crystalline state. This could mean that it is either a phase separated amorphous state or more likely, molecularly dispersed throughout the polybenzoxazine networks.

Rebinding of Template and Template-like Molecules. The MIP property of the polybenzoxazine framework can be demonstrated by considering the rebinding with a series of template as cholic acid and the model molecules, which have the functional groups to form hydrogen bond or the structure resemble to that of the template, such as deoxycholic acid, benzoic acid, carbaryl, chloramphenicol, and pyridine. Figure 9 shows the percent of binding with the template-like molecules. The binding with cholic acid is the most significant (~ 18%) as comparing to other molecules. This implies that the MIP framework of polybenzoxazines has the structure selectivity and binding sites for cholic acid, which is the original template molecule. However, the low rebinding percentage (2%) was unexpected in the case of deoxycholic acid. This might be due to the less number of OH group in deoxycholic acid to affect the hydrogen bond and the specific structure in the MIP network.

In the case of benzoic acid, the binding percentage is at 13%. This might be due to the fact that benzoic acid has a carboxylic group for binding similar to that of cholic acid. The size of benzoic acid molecule might not be appropriate for the MIP network resulting in lower binding percentage as compared to that of cholic acid. However, due to the small size, the binding might have been achieved at many positions in the MIP network. In the cases of other template-like molecules, such as

chloramphenicol, and carbaryl, the binding was not observed. For pyridine, there might be some hydrogen bonding OH---N to stabilize the pyridine ring in the structure similar to the case reported by Kirsch et al.[16]

Conclusions

Polybenzoxazine has been previously reported for host-guest properties.[14] The present work exhibited a potential application to develop a molecular imprinting polymer by a simple process of mixing and curing. Bisphenol A-based benzoxazine monomers and cholic acid were used as starting materials for the ring opening reaction to create a framework under the optimum condition (at 190°C for 8 h *in vacuo*). Cholic acid was stabilized in the framework while the removal of cholic acid from the framework was done by refluxing in good solvent overnight. The rebinding ability of the polymer demonstrated that the polybenzoxazine performed molecular imprinting to recognize the template molecule. Polybenzoxazine was demonstrated to be a molecular imprinting polymer with the specific binding sites and structure to interact with cholic acid as a template.

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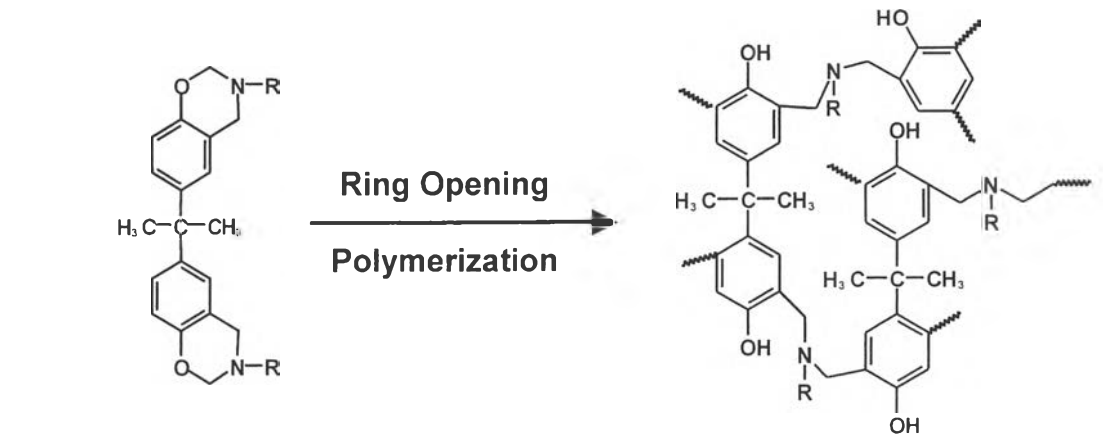
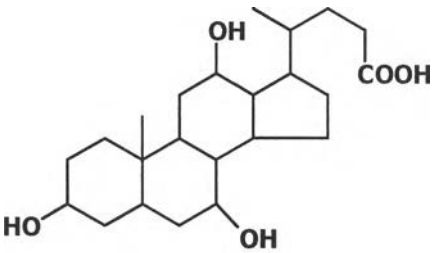
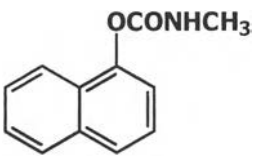


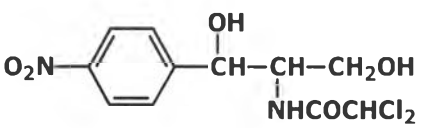
Scheme I (Chatchai et al.)



Cholic acid



Carbaryl



Chloramphenicol

Scheme II (Chatchai et al.)

Figure Captions

Figure 1. FTIR spectra of, (a) **1**; and **1** under the curing at, (b) 130; (c) 150; (d) 170; and (e) 190°C.

Figure 2. DSC thermograms of **1** under the curing at, (a) 130; (b) 150; (c) 170; and (d) 190°C.

Figure 3. DTA thermograms of template molecules, (a) cholic acid; (b) carbaryl; and (c) chloramphenicol.

Figure 4. FTIR spectra of, (a) chloramphenicol; (b) chloramphenicol after curing at 190 °C; and (c) curing product of **1** mixed with chloramphenicol.

Figure 5. FTIR spectra of, (a) cholic acid; (b) cholic acid cured at 190 °C; (c) curing product of **1** mixed with cholic acid; and (d) curing product of **1** mixed with cholic acid after refluxing in MeOH.

Figure 6. DSC thermograms of, (a) **1**; and (b) curing product of **1** mixed with cholic acid.

Figure 7. FTIR spectra of curing product of **1** mixed with cholic acid, (a) before extraction; and (b) after extraction in MeOH.

Figure 8. DSC thermograms of curing product of **1** mixed with cholic acid, (a) before extraction; and (b) after extraction in MeOH.

Figure 9. Rebinding percentage of template molecules by polybenzoxazines.

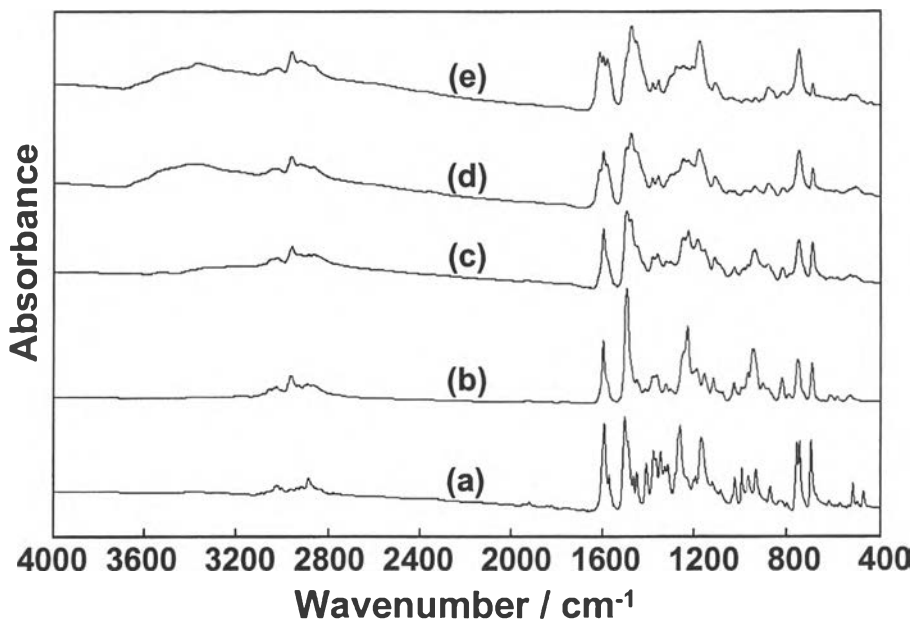


Figure 1. (Chatchai et al.)

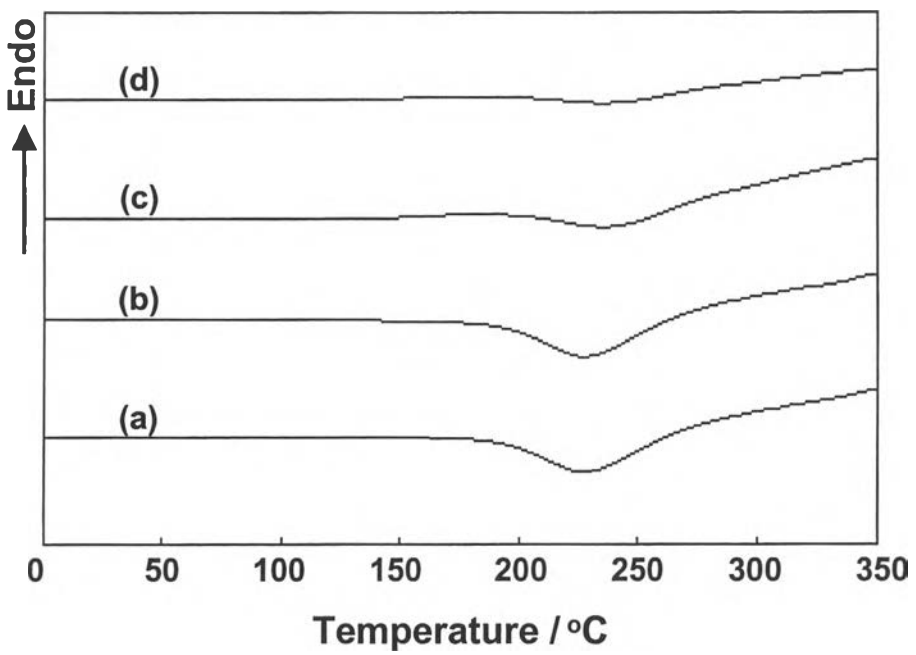


Figure 2. (Chatchai et al.)

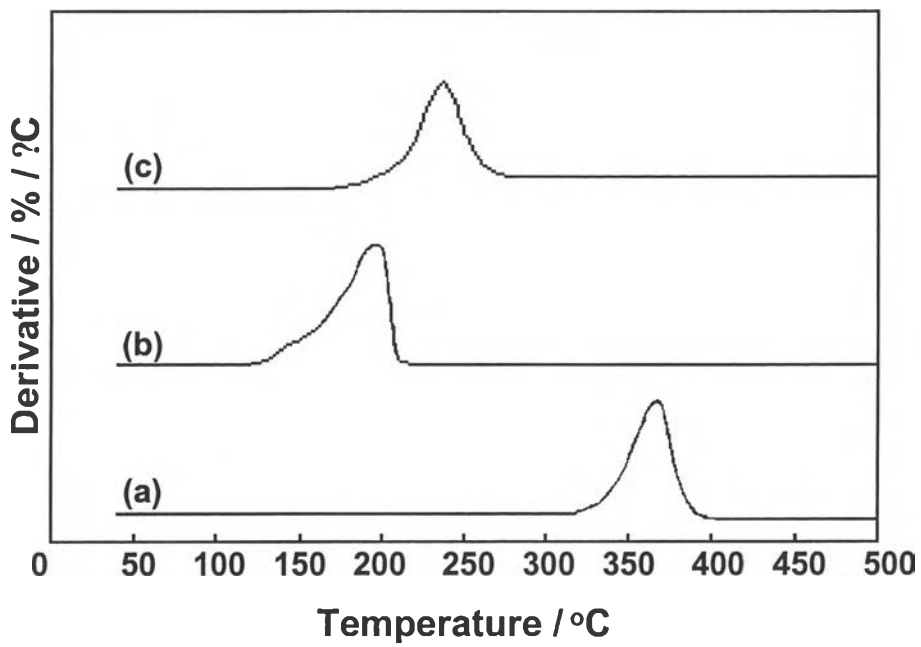


Figure 3. (Chatchai et al.)

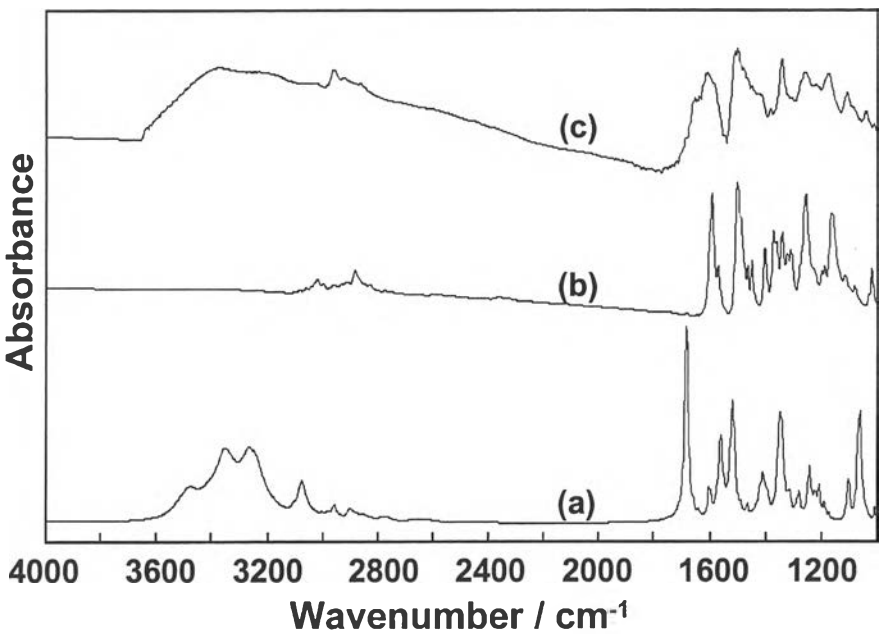
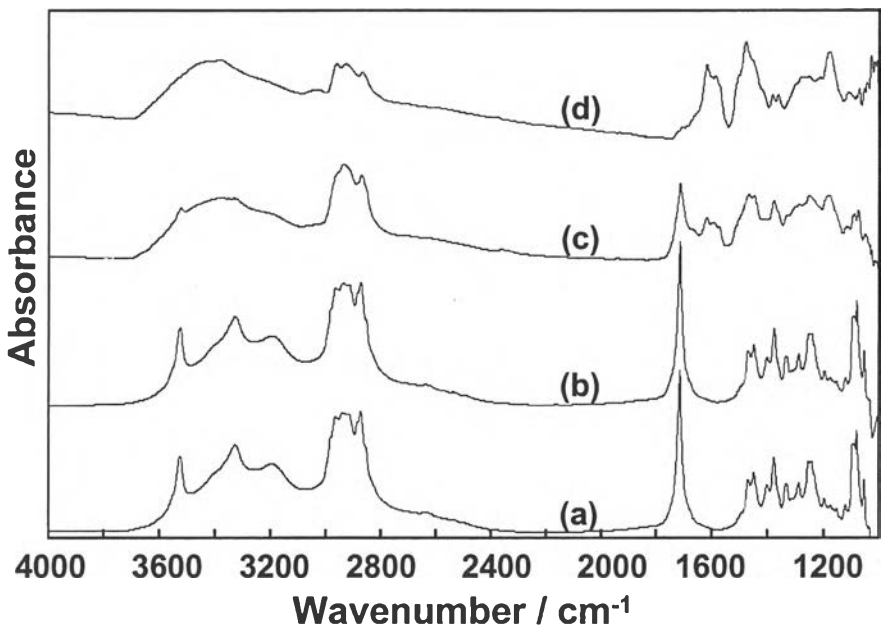


Figure 4. (Chatchai et al.)



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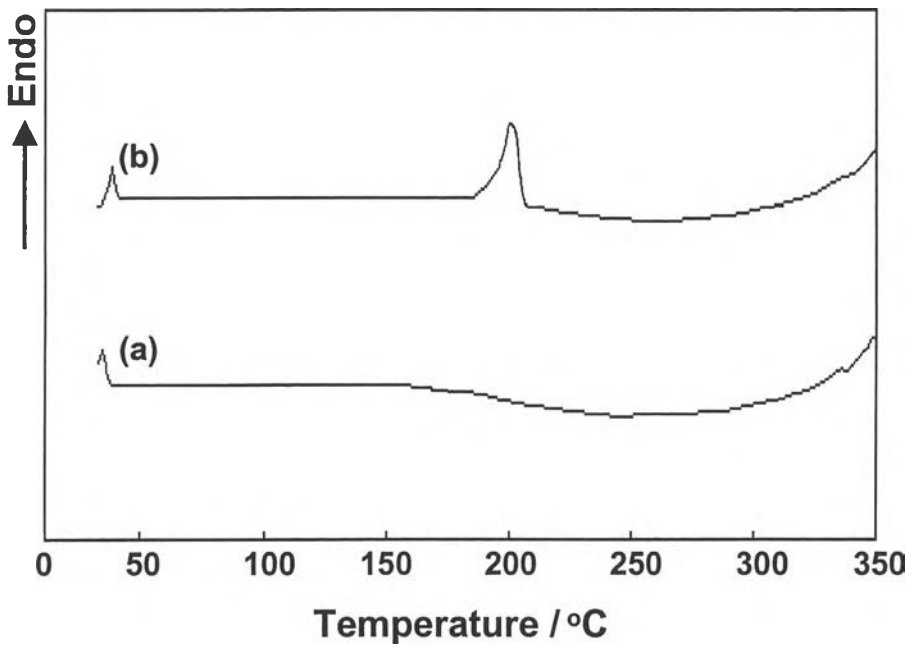
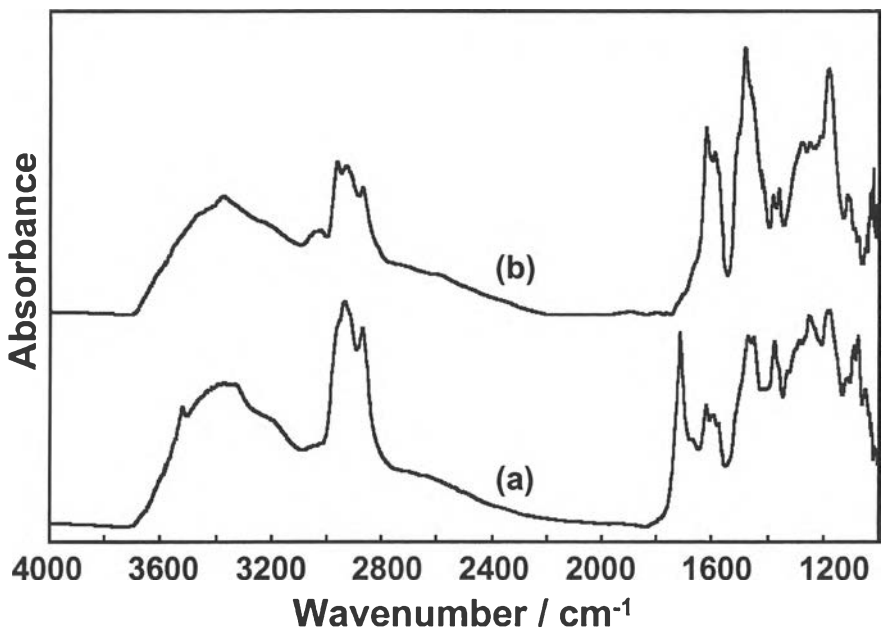


Figure 6. (Chatchai et al.)



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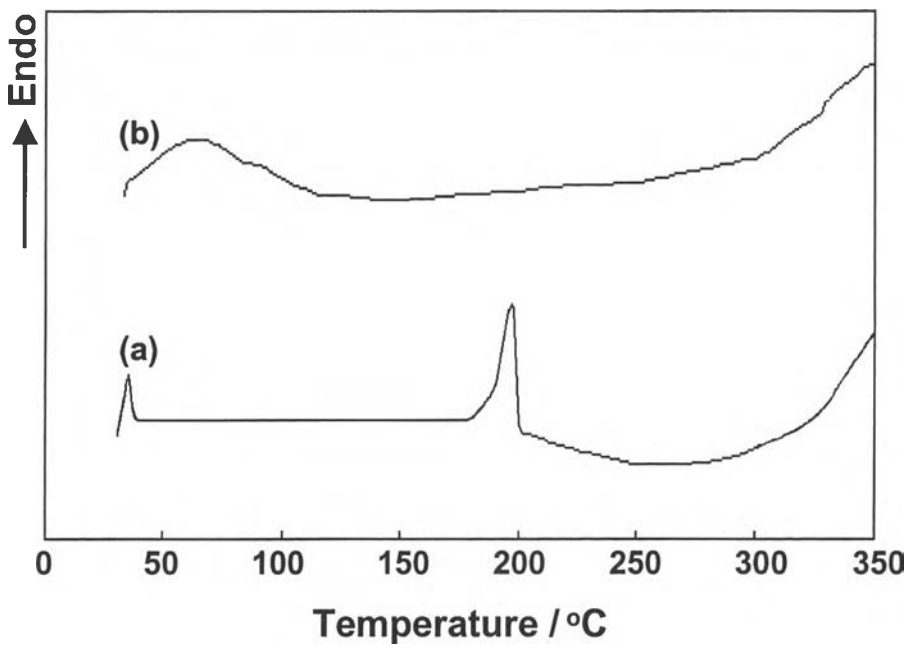

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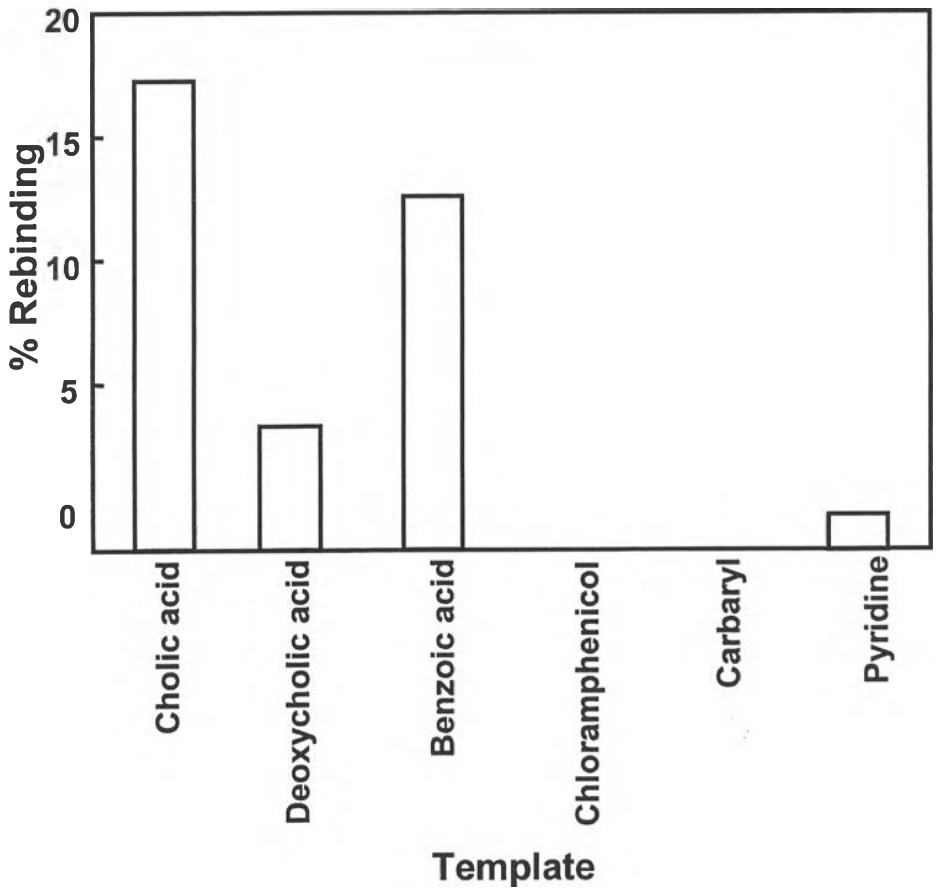

Figure 9. (Chatchai et al.)