Surface characterization and Molecular Dynamics of Nandi flame seed cuticle

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Surface Probe Microscopy and Fourier Transform Infrared (FTIR) spectroscopy have been used to characterize Nandi flame Seed Cuticles (NFSC). The FTIR has revealed that cuticles are predominantly composed of Pectin and Cellulose. The surface analysis using Atomic Force Microscopy (AFM) has shown that the cuticles are porous with average pore size of 0.5nm. The cuticles also have highly oriented topography with pores concentrated within cavities that are spaced at regular intervals. Other regions showed intertwined ridges. Isochronal and Isothermal loss plots of Dielectric Spectroscopy (DS) studies displayed two dynamical molecular processes that are strongly dependent on temperature and frequency. Differential Scanning Calorimetry (DSC) gave a glass transition temperature of 54.5°C and crystallization temperature, (T_c) of 352.4 °C. The cuticles were found to be resistant to common organic solvents.

Key Words: Nandi flame, biomaterial, molecular dynamics.

INTRODUCTION

The search for novel materials for use in packaging, nanotechnology and environmental processes has led to a plethora of studies on biological molecules and other self assembled structures (Lehn, 1990). Several examples of natural nanodevices which form excellent precursors in the design of artificial devices of the future have been reported (Di Felice and Danny, 2006). Biological materials, especially biopolymers have the
advantages of being biodegradable and from their self-assembly mechanism, complex objects can be derived (Lehn, 1990).

Biological self-assembled systems such as proteins specifically ribosomes are fascinating cell components which represent prototype for realizing biomimetic bottom-up strategies for the self-assembly of functional devices using programmed molecular building blocks (Lodish et al., 2000; Amobilino and Stoddart, 1995). Rotaxanes and Catenames have been used as natural switches (Amobilino and Stoddart, 1995). Catenane is essentially constituted of two interlocked rings and can work as a molecular switch (Amobilino and Stoddart, 1995). There is a lot of interest and effort to construct other kinds of devices based on biological molecules. In particular, self-assembling devices that express an electrical functionality are pursued in view of the development of molecular electronics (Braun et al., 1998; Keren et al., 2002; Keren et al., 2003). Recently, Ju Young et al. (2007) synthesized Urethane Acrylate Nonionomer (UAN) polymer nanoparticles which are used as absorbent for soil and water cleaning. Biopolymers with superior mechanical properties are also fascinating materials for use in packaging due to their biodegradability and renewable nature.

In this report, we present results of Atomic Force Microscopy (AFM), and Dielectric Spectroscopy (DS) of the Nandi Flame Seed Cuticles (NFSC). Composition and functional groups in the NFSC have been investigated using Fourier Transform Infrared (FTIR).

EXPERIMENTAL

Materials

Nandi Flame Seed Cuticles (NFSC) used in this study were obtained from Nandi flame trees predominantly used as an ornamental tree in Kenya.
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(Birnie, 1997). The cuticles which hold flat seeds are seasonally dispersed from mature fruits pods that split on the ground into 2 boat-shaped valves, releasing many flat-winged seeds. Figure 1 (a) and (b) show the fruit pods and the seed cuticles respectively.

Figure 1 (a): Fruits pods (b): Seed with its cuticles obtained from Nandi flame tree.

Sample preparation and characterization

Nandi flame seed was cut out from the cuticle to obtain thin cuticle films. The films were then tested for solubility in solvents of varying polarity. This was done by immersing the films in the solvents for a period of 5-14 day at room temperature. The solubility was further tested using cuticle powder. The solubility of the cuticles is shown in table 1. Samples for FTIR were prepared as pellets using Potassium Bromide (KBr) and compressing the ground material into a transparent wafer. FTIR spectra were obtained using Hyper model 8400 series spectrometer. Glass transition temperature \((T_g)\), crystallization temperature \((T_c)\) and melting temperature \((T_m)\) of NFSC were determined using a Mettler DSC 30 instrument at a scanning rate of 10K/min. for both heating and cooling runs.
Table 1: Results of the solubility test of NFSC in solvents of different polarity.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility of NFSC in natural state and in powder form</th>
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<tbody>
<tr>
<td>Diethyl ether</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Insoluble</td>
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<tr>
<td>Dimethyl sulfoxide (DMSO)</td>
<td>Insoluble</td>
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<tr>
<td>Petroleum ether</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Methyl benzene</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Benzene</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Tetrahydrofuran (THF)</td>
<td>Insoluble</td>
</tr>
</tbody>
</table>

Atomic-Force Microscopy (AFM) was used to characterize the surface of the samples in their natural state. The samples were cleaned with acetone, cut into regular shapes of surface area 0.5 cm$^2$ and then placed under the tip of the AFM. AFM has an atomically sharp tip that is attached to the terminus of a highly sensitive micro fabricated cantilever (with a spring constant in the range of 0.1–10 N m$^{-1}$, less than the equivalent constant between atoms.
in a solid). The tip of the AFM moves on the surface and generates three-dimensional (3D) images. As the probe tracked across the surface, the forces it experienced were translated into vertical deflections of the lever. These movements were in turn monitored with high accuracy by reflecting a laser beam off the back surface of the lever onto a sensitive photodetector; a typical ‘beam-deflection’ system was able to detect vertical tip movements of less than 0.5 Å. By faster scanning and detecting forces at each pixel point, a high-resolution topographic map of the surface was generated. AFM scanning was done using a Digital Instruments Multimode/Nanoscope III scanning probe microscope. Taking images at the fundamental resonance frequency of the Si cantilevers (≈300kHz) while operating the instrument in the tapping mode, the height and phase images were obtained simultaneously. Typical scans speeds during recording were 0.3-1 lines/s. Figures 3 (a and b) shows the AFM surface topographic maps of NFSC.

Molecular dynamics in NFSC were studied using broad-band dielectric spectroscopy (DS). The sample films were cleaned with alcohol and annealed at 200 °C for two hours. Aluminum electrodes of circular area of 0.5 cm diameter were deposited onto both sides of the samples. The coated samples were placed between sheets of aluminum electrodes and pressed together using micrometer screw. The dielectric measurements were performed using a high resolution alpha analyzer (Novocontrol GmbH) in a broad frequency range (10⁻¹ Hz to 10⁷ Hz) and temperature range of 100K to 600K. Below 450K the temperature was controlled by a nitrogen gas heating system which covers a range from 90 to 450K with the temperature stability within ± 0.2 K. For higher temperatures the same system was used with either piped nitrogen gas or air. The resulting dielectric spectra as a function of temperatures and frequency (Figure 5a and 5b) were fitted with the
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Havriliak-Negami function,

$$\varepsilon(\omega) = \varepsilon_{\infty} + \frac{\Delta \varepsilon}{1 + (i \omega \tau_{HN})^\beta}$$  \hspace{1cm} (1)

with $\omega = 2\pi f$, $\Delta \varepsilon$ is the relaxation strength, $\tau$ the relaxation time, $\alpha$ and $\beta$ are shape parameters that characterize the width and asymmetry of the loss curve respectively, and $\varepsilon_{\infty}$ is the unrelaxed permittivity. The relaxation time $\tau_{\text{max}}$ from the frequency of maximum loss ($\omega_{\text{max}}$) is given by

$$\tau_{\text{max}} = \frac{1}{\omega_{\text{max}}}.$$  \hspace{1cm} (2)

RESULTS AND DISCUSSION

Figure 2 (a) shows the transmittance IR spectrum obtained. The spectra obtained using the KBr disk technique compares well with that obtained directly from the pure NFSC. The spectrum show characteristic absorptions corresponding to C=C $\text{w}_{\text{k}}$ at about 1540 cm$^{-1}$, C=O $\text{w}_{\text{k}}$ at around 1683 cm$^{-1}$, C=N $\text{s}_{\text{r}}$ at 2335 cm$^{-1}$, and C-N $\text{s}_{\text{r}}$ at around 1027 cm$^{-1}$. This implies that the biopolymer contains alkenes (as the backbone), ketones/ aldehydes, nitriles, and amine groups.
Figure 2a: Transmission FTIR spectrum of Nandi flame seed cuticle.

Figure 2b shows the FTIR spectra of NFSC in comparison with other biopolymers and it is noted that NFSC is mainly composed of Cellulose and Pectins.
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Figure 2b: Absorbance FTIR spectra of NFSC in comparison with other biopolymers.

Figures 3a shows that NFSC have pores that are sunken within the cavities that separate ridges. The cuticles have highly oriented surface topography with ridges which are regularly interspaced (Figure 3b). The average pores size is about 0.5nm. These pores serve as natural paths for water absorption that assist germination of the seeds and degradation of the cuticles.
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Figure 3(a) AFM map at closer scan on the surface (1 nm above the surface) of 1559 square nm.

Figure 3(b) AFM scan showing surface configuration and the tip of AFM
Figure 4 shows a DSC thermogram of NFSC from which $T_g$, $T_c$, and $T_m$ were obtained as 54.5 °C, 352.3 °C and 404.56 °C respectively. Analysis of DSC measurement involves a plot of changes of heat capacity ($C_p$) of the sample against temperature. An increase in $C_p$ occurs after the on-set of $T_g$ as the temperature of the sample rises causing an increase in the heat capacity of the sample with no formal phase change (Skoog et al., 1998). In Figure 4, $C_p$ remained constant at around 10.5 J/g deg (°C) within 140 – 275 °C before dropping to a minimum value at around $T_c$. During crystallization, the polymer chains move into very ordered arrangements releasing heat energy in the process which results to a drop in $C_p$ or heat flow to the sample placed in a DSC cell. Beyond $T_c$ the chains become disordered and start moving freely as the polymer starts to melt. This is an endothermic process leading to an increase in $C_p$. $T_m$ is thus indicated as a peak on the DSC thermogram as shown on Figure 4.

![Figure 4: DCS thermogram showing a plot of $C_p$ versus Temperature.](image-url)
Figures 5(a) and 5(b) show the frequency and temperature dependent plots of the dielectric loss $\varepsilon''$. The frequency dependent loss curves were fitted to the Havriliak-Negami function and the average relaxation time $\tau_{HN}$ obtained. The shapes of the loss curves displayed two dynamical processes which are separated by a crossover frequency or temperature. Figure 5a, reveals a faster process I at frequencies between $10^3$ Hz and $10^7$ Hz and slower relaxation process II at frequencies below $10^3$ Hz. The isochronal plot shows that the two processes are separated by a temperature $\Delta T = 50$K. The activation plot (figure 5c) shows that process II is arrhenius in nature with an activation energy $E_a = 39.8799$KJ/mole or 0.414 eV while process I obeys the Vogel- Fulcher- Tamman (VFT) scaling law for cooperative dynamics. This is given by:

$$\frac{1}{\tau} = \frac{1}{\tau_0} \exp \left[ \frac{-B}{T - T_0} \right]$$

(3)

**Figure 5(a):** A plot of dielectric Loss factor $\varepsilon''$ vs Frequency (Hz) at a frequency range of $10^{-1}$-$10^7$Hz.
Figure 5(b): A plot of dielectric Loss factor $\varepsilon''$ vs. Temperature [K] at a temperature range of 50-400K.

where $B$ is material-dependence constant describing the degree of non-arrhenius behaviour, $T_o$ is the temperature where $\tau$ diverges and $\tau_o$ is the relaxation time extrapolated to infinity temperature (Jansson et al., 2005). The VFT expression (3) is equivalent to William-Landel-Ferry (WLF) equation (Chen-Yang et al., 2006);

$$\tau = \tau_o \exp \left[ -\frac{C_1 (T - T_g)}{C_2 + T - T_g} \right]$$

(4)
which follows from free volume theory of glass transition (Hedvig, 1977). The WLF parameters $C_1$ and $C_2$ and VFT parameters are related (Kovacs, 1964);

$$C_2 = T_g - T_0$$

(5)

$$2.303C_1C_2 = B$$

(6)

WLF parameter, $C_2 = 60K$ (Katana, 1994) while figure 5(c) show that the Vogel temperature $T_0 = -6^\circ C$. Substituting these values of $T_0$ and $C_2$ into equation (5) we obtained the glass transition temperature, $T_g$ as $54^\circ C$ which compares well with $T_g$ obtained from the DSC scans (figure 4) and is also within the range of $T_g$ of most components of cellulose and other biopolymers (Wojciechowski et al., 2006; Khare and Sandeep, 2000).

Figure 5(c): Activation plot showing both the Arrhenius fit from which the calculated
activation energy was $E_a = 39.8799 \text{ kJ/mol}$ and the Vogel-Fulcher-Tammann (VFT) fit

The relaxation process II depicts localized dynamics of the primary structure in the biopolymer. It is observed that the activation energy obtained for this biopolymer is lower than activation energies for typical synthetic polymers such as polyethylene terephthalate ($0.62 \text{ eV}$), polypropylene ($0.75 \text{ eV}$) and low-density polyethylene ($0.60 \text{ eV}$) (Das-Gupta and Joyner, 1978).

At temperatures below 250K there is a salient change in the trend of the dielectric loss where a comparatively low dielectric loss with a peak at $10^5 \text{ Hz}$ and slow relaxation process was observed (Figure 5b). This could be as a result of low energy dissipation and very slow molecular motion below 300K.

The molecular dynamics of the sample shown in figure 5(b) could be attributed to the mechanical changes at the glass transition temperature $T_g$ and diffusion of molecules into the nanopores. This causes confinement of the molecules making them relatively immobile, restricted and slow in contrast to the relatively fast relaxation of the vibration and librations below $T_g$. As a result, the main chain dynamics and localized motions become the main contribution to the molecular dynamic processes.

**CONCLUSIONS**

This work has shown that NFSC is a porous biomaterial with average pore size of 0.5nm and has a highly oriented surface topography consisting of regularly interspaced ridges. The material is mainly composed of Cellulose and Pectin. Molecular dynamics within the material exhibited two processes that are strongly dependent on temperature and frequency. Solubility test has shown that the material is highly resistant to common organic solvents. The material may also be used in packaging, and in optoelectronics.
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