ON THE PIEZOELECTRIC GALACTOMANNAN-COLLAGEN CROSSLINKED FILM AND ITS APLICATION FOR LECTIN SENSOR.

Figueiró³, S.D., Moreira³, R.A., Sombra², A. S. B., Góes¹, J.C

¹- Departamento de Engenharia Mecânica-UFC, Departamento de Engenharia Mecânica – LDM, Universidade Federal do Ceará (UFC), Campus do Pici Bloco 714, Caixa Postal 12144, CEP 60455-760, Fortaleza-CE-BRAZIL jcgoes@fisica.ufc.br., ²-Laboratório de Óptica não Linear e Ciência dos Materiais (LONLCM), UFC. ³-Departamento de Bioquímica e Biologia Molecular – UFC.

This work reports the application of galactomannan:collagen films as piezoelectric biosensor for detection lectins, a protein that bind carbohydrates with high degree of specificity. The piezoelectric strain tensor element \( d_{14} \), the elastic constant \( s_{55} \), and the dielectric permittivity \( \varepsilon_{11} \) were obtained for the galactomannan:collagen (3:2 w/w) films. Resonance measurement of the piezoelectric strain constant \( d_{14} \) of galactomannan:collagen film gives 0.081 pC/N. It was observed that adsorption onto the film slightly increased the piezoelectricity to 0.099 pC/N and decrease the film resonance frequency of \( \Delta f \approx 5 \text{MHz} \). These results open the possibility of using this films in electronic devices for galactose binding lectin detection from vegetal extracts.

Introduction

Galactomannans are plant polysaccharides displaying a mannan backbone with randomly substituted (1-6) linked \( \alpha \)-D-galactosyl units, which differ in their content of galactose and its distribution along the chain. The mannose/ galactose (M/G) ratio is one of the main chemical characteristics of galactomannans and is dependent on the extraction conditions and the plant source, and determines their physicochemical properties, such solubility in water, density and viscosity of solutions [1].

Collagen, the most abundant protein of the animal kingdom, has a long history as biomaterial. We can find it in prostheses of heart valves, in artificial skins, in contact lenses and in drug delivery systems [2]. Collagen molecules (molecular weight 300.000 g/mol) are rod-like triple helices, which are 300nm in length and 1.5nm in diameter.

Soluble collagen can be prepared from tissue, such as skin, by enzyme, acid or alkali treatments. Whereas native collagen tissue posses significant strength, this strength is lost when collagen products are made from soluble collagen. These reconstituted products may therefore require chemical treatment with crosslinking agents, so as to retain adequate strength for particular applications. Glutaraldehyde is the preferred reagent in the biomedical field and has been used extensively as a crosslinking agent to protein and polysaccharide [2].

This work reports the application of galactomannan:collagen films as piezoelectric biosensor for detection lectins, a protein that bind carbohydrates with high degree of specificity.

Experimental

Preparation of the soluble collagen

The collagen was prepared by solubilization from bovine serosa after 72h of treatment under alkaline conditions in presence of salts, followed by homogenization in acetic acid solution, at pH 3.5.

Preparation of the galactomannan solution

Galactomannan was obtained by its solubilization from seed endosperms of \textit{Adenanthera pavonina} after homogenization in acetic acid solution at pH 3.5.

Preparation of galactomannan:collagen film

The galactomannan:collagen film was prepared by adding soluble collagen to the galactomannan solution in the proportion of 60% (w/w) (sample GalCol (3:2)). The blends were casted in acrylic molds and dried in laminar flow of air.

Crosslinking of films with glutaraldehyde (GA)

In fixation with GA films, pieces of 2cm\(^2\) were immersed in phosphate buffer solution 0.1% GA, pH 7.4, for 24h at room temperature. After fixation, the pieces were treated in glycine solution (0.05M glycine:0.05M borate, pH 9.2) for 10 min, washed exhaustively with water, dried in laminar flow of air.

Affinity chromatography

Affinity chromatography of lectin was carried on galactomannan:collagen film column equilibrated with 0.15M NaCl. A crude seed extract of \textit{Artocarpus incisa}, was applied to the column, and after equilibrated for 3.5h, it was drained. After removal of unbound proteins with saline solution, the adsorbed material was eluted with 0,2M D-galactose. The eluates were collected and analysed for hemagglutination activity and measure of absorbance.
at 280nm. This procedure was repeated until no hemagglutination activity was detected.

**Scanning Electron Microscopy**

The photomicrograph of galactomannan collagen films were obtained on a Scanning Electron Microscope, Philips XL-30, operating with bunches of primary electrons ranging from 12 to 20 keV, in rectangular lyophilised samples, covered with a layer of carbon of 30 nm of thickness.

**Dielectric Function Measurements**

The complex dielectric function measurements were obtained from a HP 4291A Material Impedance Analyzer in conjunction with HP 4194 Impedance Analyzer, which jointly cover the region of 100Hz to 1.8GHz.

**Results and Discussion**

The affinity chromatography results of galactomannan: collagen film column are present in Figure 1. The frutalin, a lectin galactose specific, was retained by the galactomannan: collagen matrix and was eluted after application of 0,2M galactose on the equilibrium buffer.

**Figure 1** – Affinity chromatography

Figure 2a shows the scanning electron photomicrograph of galactomannan:collagen film. In the Figure 2b we can see the coating of galactomannan:collagen film by the lectin (sample GalCol (3:2)d), revealed by the smoothing of the surface.

**Table I**: The piezoelectric strain tensor element $d_{14}$

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\rho$(kg/m$^3$)</th>
<th>$\varepsilon$($\mu$m)</th>
<th>$d_{14}$(pC,N$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>1020,62</td>
<td>67,5</td>
<td>0,079</td>
</tr>
<tr>
<td>GalCol (3:2)</td>
<td>904,09</td>
<td>51,68</td>
<td>0,081</td>
</tr>
<tr>
<td>GalCol (3:2)d</td>
<td>916,63</td>
<td>43,56</td>
<td>0,099</td>
</tr>
</tbody>
</table>

**Conclusions**

These results open the possibility of using these films in electronic devices for galactose binding lectin detection from vegetal extracts.

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**References**