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A TSM sensor investigation of low crystallinity cellulose films

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Abstract

A thickness shear mode (TSM) quartz sensor has been used to characterize the substantivity, viscoelasticity, and mucoadhesive properties of low crystallinity cellulose (LCC) films. LCC is a novel pharmaceutical excipient that has been attributed with mucoadhesive properties. Thin films of LCC were deposited onto TSM sensors by a spin coating technique. The films were treated by passing water or 1.0% w/v mucin solution (pH 3.7 or 7.0) over the surface. Changes in the mass and viscosity of the film were observed by monitoring changes in the impedance spectra of the coated TSM sensors. Scanning electron micrographs (SEMs) of each film were used to assist the interpretation of the TSM sensor data. This study showed that LCC forms highly tenacious and viscoelastic films able to withstand prolonged (approximately 1 h) exposure to both water and mucin solution. Furthermore, these results indicate that the films may have mucoadhesive properties as LCC was found to bind significant ($P < 0.05$) amounts of mucin in comparison with control measurements. Mucin binding to the LCC sensor was greater at pH 3.7 ($P < 0.05$) than at pH 7.0, suggesting that the LCC formulation is mucoadhesive under these conditions.

Keywords: Thickness shear mode sensor; Low crystallinity cellulose; Mucoadhesion; Quartz crystal microbalance

1. Introduction

Low crystallinity cellulose (LCC) is a novel pharmaceutical excipient (Banker and Wei, 1995, 1997; Wei et al., 1996) that has been used as a base for a number of dosage forms (e.g. tablets, lotions, and topical gels). A topical non-steroidal anti-inflammatory drug gel formulation, based on LCC (Table 1), is currently being developed by CeNeS Drug Delivery for the treatment of vulvo–vaginal pain, including that associated with vulvar vestibulitis (Bergeron et al., 1997; Bordman and Peipert, 1999).

The delivery of drugs by the vaginal route can benefit by the use of bioadhesive formulations to prolong the residence time of a delivery device at the mucosal surface, thereby improving bioavaili-

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Table 1
Composition of the LCC formulation

Constituent	Quantity
Hydrated LCC	30% w/w
Carbopol 974P	0.6% w/w
Glycerol	10% w/w
Lactic acid	1% w/w
Triethylacetate buffer	QS pH 5
Water	to 100% w/w

bility or prolonging the local effect. Despite their obvious utility, the development of an optimal bioadhesive formulation is hampered by the lack of rapid and robust methods for the measurement of bioadhesion. In vitro, bioadhesion is routinely measured in terms of either the maximum force of detachment or the total work required in separating the test material from the substrate. These methods are sensitive to a wide range of experimental conditions including the physical properties (i.e. hydration) of the substrate, the pH and ionic strength of the environment in which adhesion takes place, and the rate of detachment (Wong et al., 1999). Moreover, bioadhesive studies on semi-solid formulations are often based on the native state material (Jones et al., 1996, 1997) and are therefore irrelevant to the therapeutic situation, where the semisolid formulation dries to produce a film. In vivo methods are often based on a simple index of formulation retention (Mumtaz and Ch'ng, 1995) and are therefore of limited use for formulation optimization.

Some of the limitations in the current methods for investigating bioadhesion are addressed by the use of the TSM biosensor. The TSM biosensor offers a unique and valuable in-vitro method for investigating bioadhesion, as it uses a single measurement to investigate simultaneously the mass adsorption and viscoelastic behaviour of a material at a mucous surface. The TSM biosensor is therefore highly suited to the characterization of the substantivity of topical creams, such as cellulose lotions, and the interaction of these materials with other substances, such as mucus.

The TSM quartz sensor is becoming widely recognized within the electrochemical (Ramirez and Hillman, 1998) and biosensor communities (Cavic et al., 1999) as a sensitive approach for studying mass adsorption (Sauerbrey, 1959) and material properties (Bandey et al., 1997). Film mass is measured by interpreting the shift in resonant frequency of the crystal in terms of the Sauerbrey relationship (Sauerbrey, 1959), which can be simplified to (Kanazawa and Gordon, 1985):

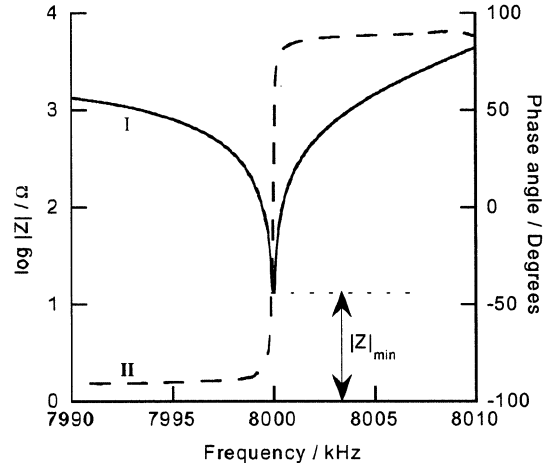

Fig. 1. Impedance spectrum of the series resonant peak for a blank TSM sensor. The spectrum indicates the relationship between impedance magnitude ($|Z|$, shown by I) and phase angle (shown by II). The series resonant frequency is determined as the point at which the phase angle is zero and the impedance magnitude is at a minimum.

$$\Delta f = -2.26 \times 10^{-6} f_0^2 \Delta m \quad (1)$$

where Δf is the frequency shift (Hz), f_0 is the original frequency (Hz), and Δm is the mass per unit area (g cm^{-2}).

Changes in the physical nature of the film are interpreted from changes in the shape of the impedance spectra (Fig. 1) of the biosensor (Bandey et al., 1997). Blank, uncoated resonators give rise to narrow resonance peaks with a low minimum impedance ($|Z|_{\min}$), which is unchanged if a rigid film is coated onto the sensor. A resonator coated with a viscoelastic film will give a broad resonance peak and a high value of $|Z|_{\min}$ relative to the uncoated sensor.

Table 2
Mass loss of LCC films on exposure to water

Initial mass of LCC layer (μg cm^{-2})	Mass of LCC layer after exposure to water (μg cm^{-2})	Mass of LCC lost(μg cm^{-2})	Percentage of LCC lost
90	80	10	11
115	107	8	7
115	108	7	6
82	81	1	1
Mean	–	–	6
Standard deviation	–	–	4

2. Materials and methods

An accurate dilution of hydrated low crystallinity cellulose placebo cream (CeNeS Drug Delivery, Table 1) was prepared by adding 240 μl of purified water (Resistivity > 17.5 MΩ cm) to 60 mg of cream, followed by thorough mixing with a glass rod.

Impedance spectra of each sensor were obtained using a Hewlett-Packard E5100A network analyser, and PI network fixture. The network analyzer was interfaced to a personal computer using a custom HP VEE 4.0 software program. Sensor frequency and $|Z|_{min}$ were determined by the software from the impedance spectra and recorded for analysis.

TSM sensors (Cambria scientific) were cleaned routinely by placing each sensor, sequentially, into piranha solution (hydrogen peroxide 40 vols: concentrated sulphuric acid, 1:3), diethyl ether, and acetone (for 4 min in each solvent). The crystal was rinsed using purified water (resistivity > 17.5 MΩ cm) after each cleaning step. After cleaning the crystal was dried in air.

Thin LCC films were produced on the TSM sensors by a spin coating technique. The sensor was spun and a 10 μl drop of cream placed in the centre of the sensor electrode, using a variable volume pipette. The TSM sensor was then spun for a further 20 s following the deposition of the LCC. Sensors were characterized by impedance analysis before and after coating, and placed in a purpose-built flow cell.

Purified water (resistivity > 17.5 MΩ cm), or 1.0% w/v mucin solution, were passed over the surface of blank and LCC coated TSM sensors. Mucin was adjusted to pH 7.0 by the addition of 0.1 M sodium hydroxide, or adjusted to pH 3.7 by the addition of 0.1 M lactic acid. All solutions were sonicated prior to use to reduce dissolved gasses. A Hayward apparatus 22 syringe driver was used to flow the solutions over one side of the crystal, at a constant rate of 0.2 ml min^{-1}, for 1 h at 20°C. The sensors were removed from the flow cell, dried at room temperature and characterized by impedance analysis. The change in film mass on coating the TSM sensor with LCC, and on exposure of the sensor to mucin and water was calculated from the shift in the sensor impedance spectra using Eq. (1). The minimum impedance value was also determined from the spectra and used to interpret changes in film rigidity.

Scanning electron micrographs (SEM) of the coated TSM sensors were taken on completion of the experiment using a Leica S430 scanning electron microscope.

3. Results

The reproducibility of LCC film loading using the spin coating technique was 65 ± 29 μg cm^{-2} ($n = 14$). On exposure to water, the mass loss (calculated from the frequency shift) of the LCC coated TSM sensors was 7 μg cm^{-2}, equivalent to a percentage decrease in film mass of $6\% \pm 4$ (Table 2). The viscoelasticity of the films is inferred from values of $|Z|_{min}$ obtained from the impedance spectra. The reported $|Z|_{min}$ values (Table 3 and Fig. 5) are given as the $|Z|_{min}$ value for the coated sensor minus the $|Z|_{min}$ for the blank sensor. Table 3 indicates that LCC films

Table 3
Rigidity of LCC films (as demonstrated by $|Z|_{min}$) before and after treatment with water

Adjusted $\|Z\|_{min}$ of coated sensor	Adjusted $\|Z\|_{min}$ of treated sensor
437	456
73	364
209	313
51	102

became less rigid after exposure to water (indicated by an increase in $|Z|_{min}$).

Statistical analysis was performed, where required, using Mintab™ (Version 14) statistics program. An Anderson–Darling test was applied to all data sets to ascertain whether the data was non-parametric ($P < 0.05$). All the data, which was statistically analyzed in this study was found to be parametric. Two sample t-tests ($P < 0.05$) were therefore used to draw comparison between data sets.

The frequency shift and mass change of both the LCC coated and blank TSM sensors before and after exposure to 1.0% mucin solution (adjusted to pH 3.7 and 7.0) is presented in Table 4. There was no statistical difference ($P > 0.05$) between mass deposited onto the blank and LCC coated TSM sensors on exposure to pH 7.0 mucin solution. However, at pH 3.7 significantly more mucin was bound to the LCC coated sensor than to the blank sensor. Impedance data for the TSM sensors on exposure to mucin solution is presented in Table 5. The reduction in the $|Z|_{min}$ values on exposure of the LCC coated sensors to mucin solutions demonstrates that there was an increase in the rigidity of the film.

Table 4
The frequency shift and associated mass increase of LCC coated and uncoated TSM sensors exposed to 1.0% mucin (pH 7.0 and 3.7)

Coating/ mucin pH	Frequency shift of mucin deposition (Hz)	Average frequency shift (standard deviation)	Mass of mucin deposited (μg cm^{-2})	Average mass (standard deviation)
LCC coated/ pH 7.0	−1629	−1729 (613)	11	12 (4)
	−2472		17	
	−1373		10	
	−2206		15	
	−964		7	
Blank/pH 7.0	−2709	−2236 (412)	19	15 (3)
	−1624		11	
	−2065		14	
	−2433		17	
	−2349		16	
LCC coated/pH 3.7	−3873	−3910 (1035)	27	27 (7)
	−4701		33	
	−4120		29	
	−4682		33	
	−2172		15	
Blank pH 7.0	−2007	−2004 (488)	14	14 (3)
	−1374		10	
	−1771		12	
	−2176		15	
	−2692		19	

Table 5
The change in rigidity, as shown by $|Z|_{min}$, for LCC coated and uncoated TSM sensors following exposure to mucin (pH 7.0 and pH 3.7)

Coating/mucin pH	Adjusted $\|Z\|_{min}$ of coated sensor	Average $\|Z\|_{min}$ (standard deviation)	Adjusted $\|Z\|_{min}$ of treated sensor	Average $\|Z\|_{min}$ (standard deviation)
LCC coated/pH 7.0	63	126 (73)	4	10 (5)
	226		17	
	130		8	
	48		9	
	161		13	
Blank/pH 7.0	0	0 (0)	0	0 (2)
	0		−1	
	0		0	
	0		0	
	0		3	
LCC coated/pH 3.7	117	89 (47)	8	28 (30)
	67		43	
	154		73	
	32		11	
	73		4	
Blank pH7.0	0	0 (0)	0	4 (7)
	0		17	
	0		0	
	0		2	
	0		0	

Figs. 2–5 show scanning electron micrographs (SEMs) of LCC coated sensors, the darker areas in the micrographs are the coated LCC film, lighter areas are the surface of the TSM sensor. Fig. 2 is a micrograph of a LCC coated sensor, which has not been exposed to either water or mucin prior to being imaged. The SEM in Fig. 3 is that of a LCC coated TSM sensor, after exposure to water in the flow cell. Figs. 4 and 5 are SEMs of LCC coated sensors after exposure to 0.1% w/v mucin that has been adjusted to pH 3.7 or pH 7.0 respectively.

4. Discussion

The coating of the TSM sensors with LCC provided a biosensor with which the physical properties and interactions of LCC with mucin could be characterized. The thin LCC films produced by spin coating were well suited to the TSM sensor technique employed in this study. The small differences between the calculated

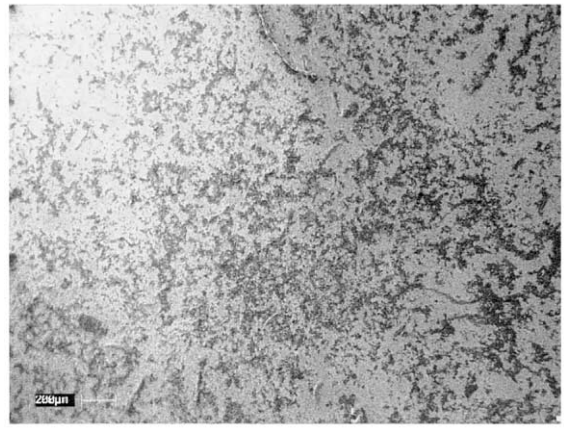

Fig. 2. SEM of LCC coated TSM sensor prior to any further treatment.

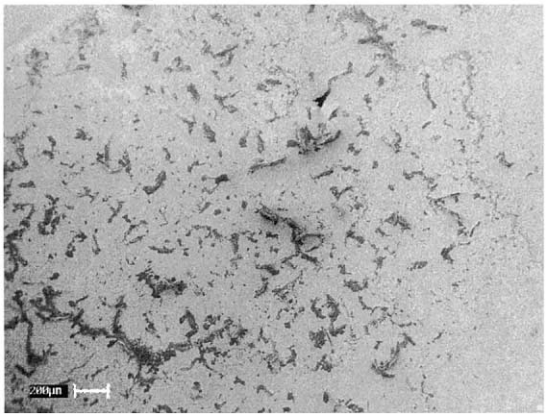

Fig. 3. SEM of LCC coated TSM sensor after exposure to water.

masses of the films (64 ± 29 μg) that were spin-coated onto the TSM sensor were due to uneven distribution of the film on the sensor electrode and small differences in the amount of film deposited. TSM sensors have a radial mass sensitivity (Ward and Delawski, 1991), i.e. the centre of the electrode is more sensitive to mass deposition than the outer edges. Therefore any non-uniformity in the distribution of the film on the sensor surface will effect the frequency shift of the impedance spectra and therefore the predicted mass of the film.

Characterization of the untreated LCC films by analysis of the impedance spectra showed viscoelastic behaviour prior to treatment with water (Table 3) or mucin (Table 5). The $|Z|_{\min}$ of the sensor (Table 3 and Fig. 5) was normalized to zero using the blank sensor value as a reference, when the LCC film was coated onto the sensor the $|Z|_{\min}$ increased demonstrating dampening of the sensor by the film. This dampening of the sensor response inferred that the film was no longer behaving rigidly. The viscoelastic nature of the film demonstrated a degree of flexibility within the chains of the LCC, despite the polymer having been dried onto the sensor surface. It is likely that the other constituents of the formulation (Table 1) plays some part in this flexibility, a high proportion of glycerol is included which could be having a plasticizing effect on the film allowing movement between the polymer chains.

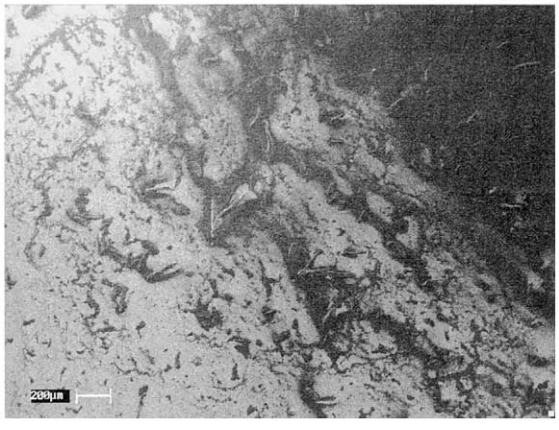

Fig. 4. SEM of LCC coated TSM sensor after exposure to mucin (pH 3.7).

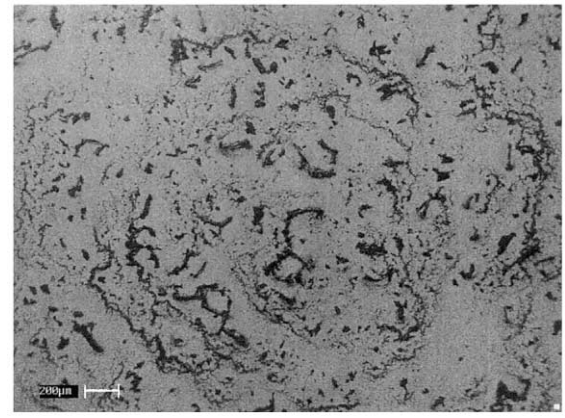

Fig. 5. SEM of LCC coated TSM sensor after exposure to mucin (pH 7.0).

The frequency shift (Table 2) of the TSM sensor indicates that there was a small decrease in frequency after 1 h treatment with water (flow rate 0.2 ml min^{-1}). This decrease was accompanied by a reduction in the viscosity of the film (apparent from the increase in $|Z|_{\min}$, Table 3). The change in $|Z|_{\min}$ of the film made precise estimation of the mass loss difficult to determine from the shift in frequency of the impedance spectra. Unlike a rigid film where the sensor and film move as if one mass, a non-rigid film causes viscous dampening of the TSM sensor causing a larger frequency response than predicted by the Sauerbrey relationship Eq. (1). If it is assumed

that the film does behave as a rigid mass the average loss of film would be $6\% \pm 4$, so if the spread of the results and the error in calculation are taken into consideration the actual loss of LCC film is likely to be very small.

While the loss of the LCC film was very small when treated with water, examination of the electron micrographs (Figs. 2 and 5) confirms that some LCC was lost. Fig. 2 shows that the untreated LCC film has a higher surface density of (dark) LCC deposits than the treated film (Fig. 3) in which more of the (light grey) electrode surface can be seen. In addition there was a loss of the smaller LCC agglomerates from the TSM sensor surface following exposure to water. In Fig. 2, the LCC film (dark grey areas) appears to consist of small discrete particles, whereas on the water treated film only the larger LCC agglomerates remain. The selective removal of the smaller particles was probably due to faster dissolution of the higher surface area deposits. Whether the same trend occurred when the LCC films were treated with mucin (Figs. 4 and 5) was more difficult to determine, as the LCC film was almost indistinguishable from the mucin deposits. However, these films do appear to have less of the small LCC agglomerates than the untreated film. Closer examination of the untreated and water treated LCC agglomerates at higher magnification (figure not shown) revealed that the film had a more amorphous appearance after treatment with water. This correlates with the results from the TSM sensor indicating that the film became more viscoelastic after exposure to water.

Exposure of both the coated or uncoated TSM sensor to 1.0% w/v mucin resulted in a decrease in the frequency shift of the sensor (Table 4). This trend was opposite to that for water, and demonstrated that mucin was binding to both the LCC film and the uncoated hydrophilic gold electrode of the sensor. The frequency shift when mucin, adjusted to pH 7.0, was passed over the LCC coated sensor corresponded to an average change in film mass of 12 μg cm^{-2}. Analysis using a two sample t-test ($P < 0.05$) indicated that this change was not significant when compared to the mass of mucin absorbed to the blank sensor.

The TSM and SEM results for mucin at pH 3.7 suggested that LCC may possess mucoadhesive properties at this pH. When 0.1% w/v mucin solution was adjusted to pH 3.7, the frequency shift of the LCC coated TSM sensor (Table 2) exhibited significantly higher mucin binding than for either mucin at pH 7.0 or for the pH 3.7 control measurements (two sample t-test, $P < 0.05$). On average, approximately twice the amount of mucin was bound to the LCC coated sensor at pH 3.7 than at pH 7.0 or the control. The increased mucin binding was also seen on the SEM images. Comparing SEM images of the LCC coated sensors exposed to mucin at pH 3.7 (Fig. 4) and pH 7.0 (Fig. 5) clearly shows that more mucin was bound to the surface of the pH 3.7 sensor. Dependence of mucoadhesion on solution pH has been reported previously (Lehr et al., 1992) for polymer films. Carbopol, a minor constituent of this formulation (Table 1) has been shown to have a higher detachment force at low pH than at high. It is highly likely that the carboxyl groups of the carbopol and lactic acid constituents of this formulation are responsible for the pH dependent mucoadhesion. Mucin has an isoelectric point of pH 3–5, carbopol a pK_a of 6, and lactic acid a pK_a of 4. At pH 7.0, mucin will have a net negative charge leaving little opportunity for interaction with the negatively charged carboxyl groups of carbopol and lactic acid. When the pH of the mucin solution is lowered to pH 3.7 the net charge of the mucin will be positive, allowing binding of the mucin to the weakly ionized carboxyl groups of the LCC formulation.

Despite the differences observed in the frequency shift of the TSM sensors, similar impedance spectra were obtained for mucin at pH 3.7 and at pH 7.0. At both pHs the $|Z|_{min}$ of the TSM sensor (Table 5) increased on coating the LCC film and decreased again on exposure of the film to mucin. This showed that before treatment the film possesed a degree of flexibility, and that on exposure to mucin this flexibility disappears and the film becomes rigid again. Possible explanations for the change in film rigidity include structural changes within the formulation, alterations in sensor/film binding, or mucin/formula-

tion binding. It seems unlikely that an alteration to the polymer structure of the formulation has occurred on exposure of the LCC film to mucin solutions of either pH or that any change in the binding to the sensor has occurred. LCC films were unaffected by exposure to water and mucin lacks the chemical reactivity to catalyze changes in the binding between polymer chains. A much more plausible explanation for the change in film rigidity on exposure to the solution is that mucin has been bound to the LCC film. It is evident from the measurements made using blank TSM sensors that mucin behaves as a rigid layer (Table 5). Deposition and binding of the mucin onto the LCC film probably rigidifies the system preventing movement of the polymer chains in the LCC formulation.

5. Conclusions

Coated LCC films consist of discrete, viscoelastic particles of the formulation; the average mass of film deposited using the spin coating method is 65 μg $cm^{-2} \pm 29$. The results suggest that the LCC films are highly tenacious i.e. resistant to removal by water and 0.1% mucin solution over a 1 h period. Mucin (both at pH 3.7 and pH 7.0) adsorbs onto the sensor causing an increase in rigidity of the film. The adsorption of mucin (at pH 7.0) onto the coated and uncoated sensor is similar to that for mucin binding to the uncoated sensor. However the adsorption of mucin to the coated sensor is significantly greater at pH 3.7 suggesting that the LCC film is mucoadhesive under these conditions.

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