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International Journal of Pharmaceutics 195 (2000) 25–28

international journal of pharmaceutics

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Communication

Characterisation of fatty acid multilayers using a TSM biosensor☆

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Received 18 August 1999; received in revised form 7 September 1999; accepted 11 October 1999

Abstract

Thickness shear mode (TSM) biosensors have many potential applications within the pharmaceutical sciences as a means of measuring mass changes in the nanogram range, film thickness, viscosity and shear moduli. This study addresses the possible use of the TSM sensor as a biosensor for measuring drug partition coefficients. In order to realise this potential, some fundamental understanding is required of the behaviour of lipid films on the sensor. The present study characterises the behaviour of fatty acid multilayers as a suitable model chemical system. Frequency shifts and impedance spectra are presented for multilayers of three fatty acid films coated on to the sensor using a Langmuir-Blodgett trough. The results indicate that the frequency shift is non-linear at lower numbers of fatty acid layers but the response is Sauerbrey-like at higher numbers of layers. Also at high numbers of layers, changes in the impedance spectra indicate viscoelastic behaviour in thicker membranes. An inverse relationship is observed between chain length and frequency shift, which is attributed to variations in the topography of the sensor surface. This work demonstrates the importance of fully characterising the physical behaviour of the lipid multilayers prior to using these systems for the measurement of drug partition coefficients. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Thickness shear mode sensors; Biosensor; Fatty acid; Partition coefficient

1. Introduction

Thickness shear mode (TSM) sensors can be used in two ways: either as a quartz crystal microbalance (QCM) capable of studying nanogram changes in the mass, or coupled to an impedance analyser to determine the viscosity and/or elasticity of films. The QCM is restricted to the study of rigidly coupled solid films, thus limiting its use in biosensor applications where the biological substrate is likely to exhibit some non-ideal behaviour. Impedance analysis of TSM sensors,

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☆ Presented in part at UKaps Annual Conference, Manchester, UK, 28–30, June 1999.

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PII: S0378-5173(99)00354-3

however, allows the determination of mass and mechanical changes in both Newtonian fluids and viscoelastic media and is therefore highly suited to biosensor applications.

Formation of a film on the TSM sensor (i.e. the quartz resonator) alters the intrinsic properties of the system and reduces the frequency of oscillation of the resonator. A rigid mass loaded onto the surface of the crystal will not alter the shape of the resonant peak but merely shift the peak to a lower frequency. For a rigid surface film, the change in frequency is proportional to the mass loaded according to the Sauerbrey relationship (Sauerbrey, 1959):

$$\Delta f = \frac{2nf_0^2}{\sqrt{\rho_q \mu_q}} \Delta m \qquad (1)$$

where Δf is the change in frequency (Hz), n is the number of sides coated, f_0 is the original resonant frequency (Hz), Δm is the mass change per unit area (g cm^{-2}), and ρ_q and μ_q are the density and shear stiffness of the quartz. Changes in the shape of the impedance spectra can be interpreted qualitatively or quantitatively in terms of changes in the material properties (viz. shear moduli, density, and film thickness) of non-rigid systems (Bandey et al., 1987). Viscoelastic changes within a film are indicated by a reduced frequency shift (relative to an equivalent rigid mass), a broadening of the peak and an increase in the peak impedance.

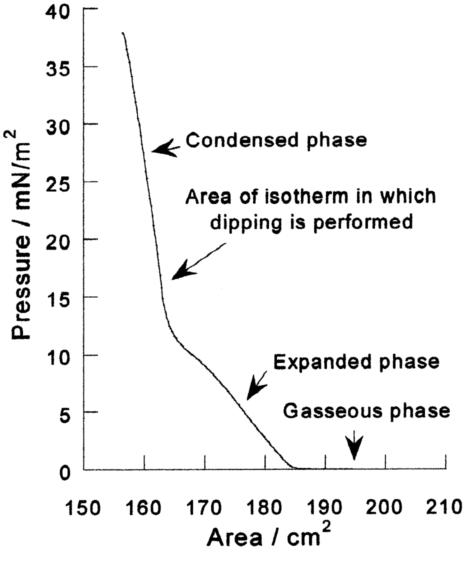

Fig. 1. Typical isotherm for arachidic acid, showing the different phase states and the surface pressures at which the TSM sensors were coated.

In this investigation, qualitative changes in the series resonant peak of impedance spectra are used as an indicator of viscoelastic change in the system.

As a method of odour sensing, Okahata and Shimizu (1987) monitored molecule partitioning into lipid membranes using the QCM technique by measuring the partitioning of organoleptic molecules into multilayer films. In this study we are using the TSM sensor to investigate the validity of the mass-frequency relationship by examining the viscoelastic behavior of model lipid systems (i.e. saturated fatty acids with chain lengths between 18 and 22). Characterisation of such lipid systems will provide the foundation for the development of a method for high throughput determination of drug partition coefficient.

2. Materials and methods

Using a Hewlett Packard E5100A network analyser and 9-MHz AT-cut quartz crystals (electrode area 0.2 cm^2), impedance analysis measurements were performed on three fatty acid films: stearic acid (18 carbon saturated fatty acid), arachidic acid (20 carbon saturated fatty acid), and behenic acid (22 carbon saturated fatty acid). Multilayers of each fatty acid were coated on to the quartz sensor using a Nima Technologies 611 Langmuir-Blodgett trough, with a 10^{-3} M zinc sulphate subphase. Coating was performed at pressures of 2–5 N m^{-2} above the condensed/expended phase interface (Fig. 1). The molecular cross-sectional area was calculated (at the dipping pressure) by measuring the area of known concentrations of fatty acid spread onto the subphase surface. After dipping, the coated crystal was dried at 35%RH until the surface subphase was removed. Impedance spectra were measured for increasing odd numbers of fatty acid layers between 1 and 13. The mass loading of the films onto the quartz resonator was observed quantitatively by the frequency shift of the series resonant peak, while changes in the viscoelastic properties

Table 1
Mean cross-sectional area for stearic acid, arachidic acid, and behenic acid Langmuir-Blodgett films ($n = 3$)

Fatty acid	Cross-sectional area of molecule, $(\mathrm{cm}^2/\times 10^{-15})$	Theoretical mass per unit electrode area of fatty acid monolayer $(\mathrm{g\ cm}^{-2})$
Stearic acid	2.0 ± 0.06	2.37×10^{-7}
Arachidic acid	1.9 ± 0.05	2.74×10^{-7}
Behenic acid	2.0 ± 0.01	2.84×10^{-7}

of the films were observed qualitatively from changes in shape of the impedance spectra.

3. Results

The cross-sectional area occupied by the fatty acid molecules, in the condensed film through which the quartz resonator was dipped, are shown in Table 1. These areas indicate that the fatty acid films all have the same degree of packing when deposited on to the TSM sensor surface. The frequency shift of the sensor on deposition of the multilayer films is shown in Fig. 2. These data show that for each lipid, a non-linear frequency shift was observed at low numbers of layers, followed by a linear increase in frequency shift at higher numbers of layers. An inverse relationship between frequency shift and chain length of the fatty acid was also observed. The theoretical mass loading for the first fatty acid layer is shown in Table 1. These theoretical masses were calculated based on the assumption that the first fatty acid layer is rigidly bound to the sensor surface and that the orientation and packing of the lipids on the film balance is replicated on the sensor surface. When compared to the uncoated sensor, impedance spectra of the coated sensor (Fig. 3) display a broadening of the series resonance peak at higher numbers of layers.

4. Discussion

Similarities in the resonant peak shape for the uncoated resonator with that for a single layer of fatty acid (Fig. 3) indicate that the film is behaving as a rigid mass. This rigid coupling enables the mass of the first layer to be calculated using the

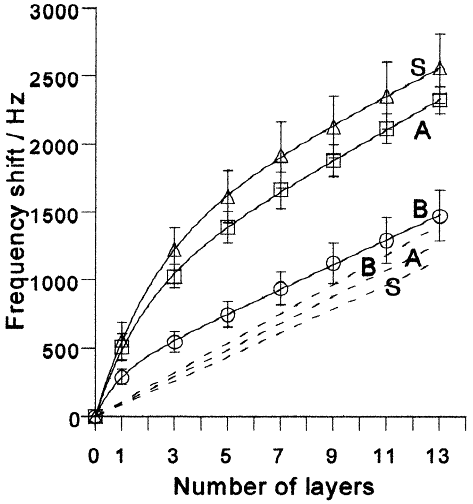

Fig. 2. The frequency shift for successive layers of three fatty acids; stearic acid (S), arachidic acid (A), and behenic acid (B) coated on to a TSM sensor ($n = 3$). Shows theoretical Sauerbrey relationships (dashed lines).

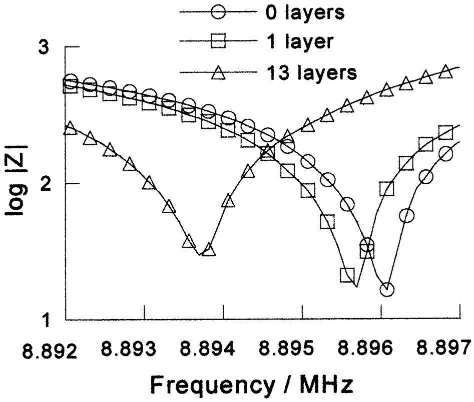

Fig. 3. Typical impedance spectra for an arachidic acid film coated on to a TSM sensor. Spectra shown are for the blank sensor, and one and 13 arachidic acid layers.

Table 2
Mean frequency shift, and film mass for the first layer of three fatty acid films ($n = 3$)

Fatty acid film	Mean frequency shift for one layer of fatty acid (Hz)	Mean mass change per unit electrode area for one layer (g cm^{-2})
Stearic acid	557	1.6×10^{-6}
Arachidic acid	507	1.4×10^{-6}
Behenic acid	291	0.8×10^{-6}

Sauerbrey relationship (Eq. (1)) and the results suggest mass loading in the tens of micrograms range (Table 2). This is unexpected and is not consistent with the theoretical mass of complete coverage ($0.2–0.3 \times 10^{-6}$ g cm^{-2}) calculated using the mean cross-sectional area of the molecules (Table 1). The discrepancy between the actual and theoretical masses is most likely explained by the entrapment of the water subphase in surface irregularities on the sensor. The quartz sensors used in this study are unpolished and therefore posses a microscopic surface roughness. It has been shown by Schumacher et al. (1985) that material can become trapped in the irregularities causing it to behave as a rigid mass. We believe that the difference between calculated and theoretical mass is a result of this effect. This belief can be confirmed by the use of polished quartz crystals.

The non-ideal response caused by the trapped water also explains the inverse relationship between frequency shift and chain length (Fig. 2). By chance, the volume of the surface irregularities on the crystals (and hence the mass of water entrapped) were ordered inversely to the chain length of the fatty acid molecules. This conclusion was supported by a study in which the crystals used for each fatty acid were swapped over. Again, the use of polished quartz crystals should negate the effect of the subphase entrapment allowing the true relationship between fatty acid chain length and mass loading to be determined.

Significantly a transition to viscoelastic behavior is indicated by the broadening of the series resonant peak when thirteen layers have been deposited (Fig. 3). As layers of fatty acid molecules are added to the surface, both intra and interlayer motion may increase, resulting in the film behaving progressively more viscoelastically at higher numbers of layers. These viscoelastic changes are likely to continue until the bulk viscoelastic properties of the lipid material is reached, at which point any further change in impedance spectra is simply due to the increasing thickness of the film. The linearity at higher numbers of layers indicates that a Sauerbrey like relationship may exist once the viscoelasticity of the system is constant (i.e. that frequency shift is a linear function of the mass and thickness of the layers added).

These results demonstrate that there is a necessity to pre-characterise the viscoelastic properties of lipid membranes before the TSM biosensor approach may be used to obtain reliable partition coefficient results.

Acknowledgements

Pfizer Central Research and De Montfort University are acknowledged for joint funding of this project.

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