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Abstract

The lung permits gaseous exchange between the body and atmosphere. The principal interchange site is the alveolar space, which is bathed in a lipid-protein blend called pulmonary surfactant. This material minimises the surface tension and maintains airway patency. Pulmonary surfactant is the initial contacting site for orally inhaled products and environmental toxins. Langmuir monolayer technology can be applied to model the alveolar space. A recent development in this field is the lung biosimulator. The aim of this study was to investigate the influence of cannabis smoke on the activity of the lung surfactant replacement product, Curosurf®. Here, the lung biosimulator facilitated controlled operating conditions of 37°C, elevated humidity and accepted fluid hydrodynamics. Initially, 50mg cannabis material was pyrolysed and the smoke collected. For complete pyrolysis, a regimen involving 4 puffs, 50ml volume, 3 second puff duration and a 30-second interval was applied. Quantification for cannabis smoke was conducted via gas chromatography – mass spectroscopy, with a mean concentration of 1% Δ9 tetrahydrocannabinol (THC) determined. Cannabis smoke aliquots were transferred to the lung biosimulator and 10 minutes allowed for interaction. Expansion – contraction cycles were then initiated to mimic tidal breathing. Baseline data confirmed that Curosurf® works effectively, under physiologically relevant conditions. High surface pressures (e.g. 70mN/m) were attained on full compression. Exposure to cannabis smoke from two independent batches increased the compressibility term and reduced the Langmuir isocycle maximum surface pressure by approximately 20%; interbatch variation was detected. Cannabis smoke impaired the ability of Curosurf® to lower the surface tension term. This was ascribed to the penetration of the planar, hydrophobic drug into the two-dimensional film and destructive interaction with polar functionalities. The net effect would be increased work of breathing for the individual.

Key words

Langmuir monolayers, pulmonary surfactant, lung biosimulator, cannabis, gas chromatography.

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1. Introduction

The cultivation and use of cannabis by mankind dates back at least 6000 years [1]. Over time, moving more towards the modern day, cannabis has attained infamy as the most widely cultivated and used illicit drug in the world [2]. Cannabis may be regarded as a generic term that is used to describe complex series of plants of which there are two main species that contain 400 different chemicals; more than 60 being compounds that are referred to as cannabinoids [3]. The popularity of this material can be directly ascribed to its psychoactive properties. Notwithstanding the popularity of cannabis, administration of the compound to the body does cause both acute and chronic health consequences. For instance, the World Health Organisation reports that acute adverse effects can include impaired cognitive function involving memory and learning [2]. In addition, apparent psychomotor effects can diminish the attention span and thus increase the risks of accidents (e.g. during the use of machines or driving motor vehicles) [2]. The chronic health implications relating to cannabis use are numerous and include schizophrenia exacerbation, further impairment of cognitive function [4] and deleterious pulmonary effects such as bronchitis [5].

The main psychoactive component of cannabis smoke is delta-9-tetrahydrocannabinol (Δ9-THC), as detailed in Figure 1. This molecule was first isolated by Gaoni and Mechoulam in 1964 [6].

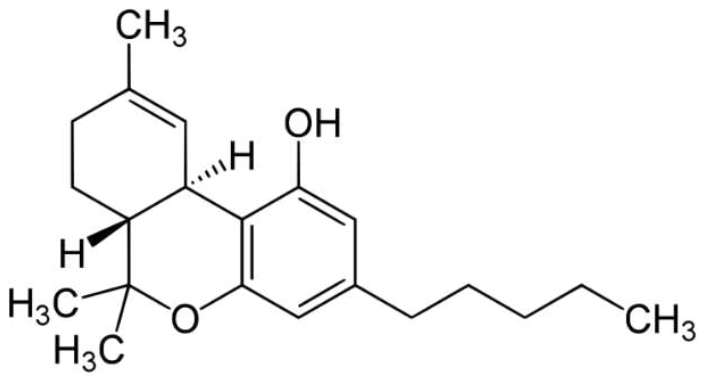

Figure 1. *The Chemical Structure of Delta-9-tetrahydrocannabinol*

Tetrahydrocannabinol is a tri-cyclic, 21-carbon molecule with low aqueous solubility and high lipophilicity [7]. The chemical nature of the molecule results in rapid diffusion across lipophilic interfaces (e.g. the hydrocarbon carpet expressed by pulmonary surfactant in the alveolar space [8] plus the membranes of type I and II pneumocytes), resulting in fast absorption into the systemic circulation. Accordingly, peak plasma concentrations are typically noted at approximately 3 - 10 minutes post cannabis inhalation [9]. With regards to key chemical aspects of Δ9-THC, the oxygen atom within the phenolic group displays a single lone pair of electrons which lie within the plane of the planar aromatic ring system and the hydrocarbon side chain. The oxygen atom integrated within the pyran ring structure houses a second lone pair of electrons which is delocalised across the region [10]. Such delocalisation causes the phenolic group to serve as a weak hydrogen bond donor. The central phenolic group is the most important polar functionality within the Δ9-THC molecule because the ether oxygen atom is sterically hindered by the two methyl groups, preventing it from acting as a strong hydrogen bond acceptor. To date, there is a paucity of data surrounding the effect(s) of cannabis smoke on airway performance, which is the principal delivery route for a systemic presence.

Pulmonary surfactant represents the initial contacting surface for inhaled material that is delivered to the airways (i.e. environmental toxins or respirable pharmaceutical formulations) [11 & 12]. This material is a complex, endogenous mixture composed of lipids and proteins that serves to reduce the surface tension at the alveolar air-liquid interface. Approximately 90% of the material is phospholipid-based [13], with the remaining 10% being proteinaceous in nature. The lipid fraction is multifaceted, demonstrating 85% phospholipids with the remainder containing cholesterol, neutral lipids, triglycerides and fatty acids [14]. The phospholipid fraction is comprised of 70-80% phosphatidylcholines [15] of which the predominant entity is dipalmitoylphosphatidylcholine (DPPC); this is the principle surface active agent within pulmonary surfactant [15]. The molecule displays a gel to liquid phase transition at 41°C, hence at physiological temperatures it exists as an ordered gel which can limit spreadability in the (deep) lung [16]. For this reason, other species such as palmitoyloleoylphosphatidylglycerol (POPG) and palmitic acid (PA) are involved in the system and act to facilitate material spreading and fluidity [17]. The proteinaceous fraction of the natural surfactant mix involves four different surfactant proteins (SP); namely SP-A, SP-B, SP-C and SP-D. The hydrophilic SP-A and SP-D molecules are the most abundant proteins within the surfactant blend and are a part of the Ca^{2+} dependent collectin family.

The SP-B and SP-C molecules are hydrophobic proteins, with the SP-B molecule having an important role in facilitation of surface adsorption, lowering of surface tension and re-spreading of surfactant film from collapsed phase [18 & 19]. The SP-B molecule has also been found to increase stability of the lipid monolayer and confers enhanced resistance to surface tension by its effect on the molecular orientation of the phospholipid monolayer [20]. The SP-C molecule is the most hydrophobic of all the surfactant proteins and its functions have not been fully elucidated but they seem to overlap those of SP-B [21].

A range of exogenous pulmonary surfactant products are available to manage respiratory distress syndrome that frequently arises in neonates [22]. One such product is Curosurf®, which is extracted from porcine lungs and purified to form a suspension comprising polar lipids (e.g. 70% DPPC), SP-B and SP-C with no additional additives [23]. The recommended dosing regimen for this product is 2.5ml / kg birth weight (which is equivalent to 200mg/kg birth weight) [24]. This preparation is composed of 80mg/ml phospholipid, of which 70% is phosphatidylcholines, and 0.9mg/ml of SP-B and SP-C [25]. The structure-function activity of surface active material can be studied within the laboratory setting via application of Langmuir monolayer technology. Here, potential exists to modify an array of environmental / operational parameters such that the test zone reflects the *in vivo* state [26]. As a result of inherent chemical properties, the amphiphilic material of interest is positioned such that hydrocarbon functionalities orientate towards the external gaseous environment and the polar head groups associate directly with the supporting aqueous subphase [8]. Once in position and equilibrated, lateral forces can be applied to the two-dimensional film under investigation either in isolation or indeed quick succession; the latter providing a good model for the human tidal breathing cycle. Typical data sets for work of this nature include Langmuir pressure-area (π-A) isotherms plus Langmuir pressure-area isocycles. Deviation within these experimental plots from the baseline (i.e. post exposure to an environmental stressor (e.g. cannabis smoke)) is indicative of a physicochemical interaction between the species under consideration. This approach has previously been successfully applied in a number of studies relating to environmental toxins [12, 27 & 28] and as such will be applied herein to investigate the impact of cannabis smoke on pulmonary surfactant function.

This study aims to probe the response of pulmonary surfactant monolayers when exposed to cannabis smoke under physiologically relevant conditions. Here, consideration will be given to the chromatographic analysis of cannabis smoke along with potential mechanisms of interaction with pulmonary surfactant that may ultimately influence the human breathing cycle.

2. Materials and Methods

2.1 Materials

During this study the commercially available lung surfactant replacement preparation Curosurf® (Chiesi Ltd, Italy. Lot: 1051267) was applied in order to represent the alveolar air-liquid interface. Due to the inherent viscosity of this product, a dilution step was incorporated before application in the lung biosimulator. Here, a buffer solution (i.e. NaCl (150mM), $CaCl_2$ (2mM) and $NaHCO_3$ (0.2mM); pH 7) was prepared to dilute the preparation to a lipid concentration of 1mg/ml [29]. At the outset, a suitable volume of the diluted surfactant replacement product was applied to the aqueous subphase contained within the Langmuir trough to increase the surface pressure to between 15mN/m – 20mN/m, as previously detailed [28]. In total, two batches of cannabis were applied during this study. The herbal material was obtained from Merseyside Police under a Home Office Research Licence and used as supplied. Chloroform ($CHCl_3$) (Sigma-Aldrich, UK) of analytical grade (≥ 99.9%) was employed to clean contacting surfaces. In terms of chemical analysis, ethanol (Analytical grade, In-house production) was employed as the solvent to facilitate chromatographic analysis. Ultrapure water (Purite, UK), demonstrating a resistivity of 18 MΩ.cm, was used for the cleaning procedures and as the Langmuir trough aqueous subphase.

2.2 *Methods*

2.2.1 Langmuir Monolayer Preparation

Surfactant monolayers were produced and held within the lung biosimulator during all work presented herein [26]. Surfactant free tissues (Kimtech Science, Kimberley-Clark Professional, 75512, UK) were soaked in chloroform and used to clean all contacting glassware and equipment surfaces. Cleanliness checks were performed on the Langmuir trough (Model 102M, Nima Technology, UK) to ascertain contamination levels ahead of data collection. Here, a surface pressure value of 0.4mN/m or less was deemed appropriate on full barrier compression. A Hamilton microsyringe was applied to deliver the amphiphilic material to the surface of the aqueous subphase. In this case, sufficient material was applied in order to reach the pre-determined starting target pressure of 15mN/m – 20mN/m. A period of 10 minutes was allowed for material spreading across the aqueous subphase of 70cm^2 surface area.

The trough barriers were programmed to move to the centre of the compartment at a rate of 25cm^2/min in the case of Langmuir isotherm generation and 100cm^2/min in the case of Langmuir isocycle generation. Plots of surface pressure vs. percentage trough area at 37°C and elevated humidity (e.g. 85% RH) were collected using a Wilhelmy plate held at the centre of the Langmuir trough.

2.2.2 Cannabis Smoke Generation

To obtain cannabis smoke from the starting material, all glassware was initially cleaned and connected then placed with a fume hood as illustrated in Figure 3. Appropriate tubing and seals enabled the preparation of an airtight system.

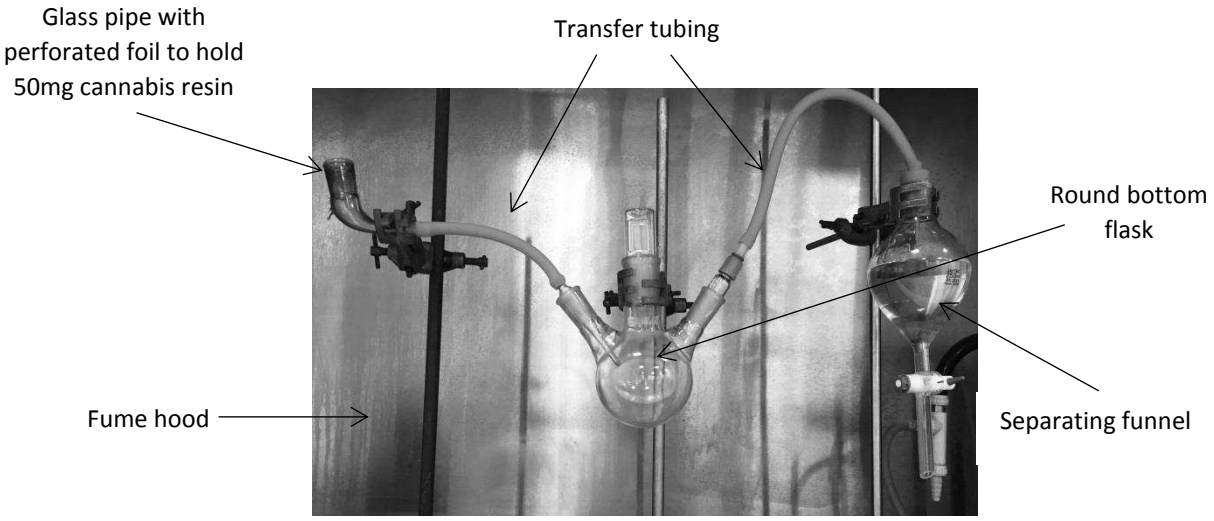

Figure 3. *The arrangement applied to collect cannabis smoke vapour aliquots.*

Before the herbal material was ignited, 200ml of water was poured into the 250ml separating funnel. In total, 50mg of the cannabis starting material was pyrolysed using a long-necked lighter and simultaneously 50ml of water was pulled through the 250ml separating funnel to create a vacuum. Smoke collected in the 250ml round bottom flask, and settled for 30 seconds. A further 50ml of water was pulled through and this process was repeated until a total volume of 200ml of water was used (i.e. 4 puffs). The rubber tubing connecting the round bottom flask was removed and stoppers inserted immediately to ensure no loss of cannabis smoke. At that stage the cannabis smoke sample could be delivered directly to the lung biosimulator. For GC-MS analysis, a volume of 1ml of analytical grade ethanol was added to solubilise the smoke aliquot.

2.2.3 Chromatographic Analysis of Cannabis Smoke

The resultant cannabis smoke samples were diluted to a 1:10 dilution and hexadecane was added as an internal standard. The analysis of the smoke samples and THC standards (10 -75 µg/ml range) was performed using an Agilent 6980GC with 5975MS detection. The column was an Agilent J&W HP5-MSUI with the dimensions of 30m x 0.250mm x 0.25mm and a split (10:1) injection of 1µl. The oven time and temperature parameters were 5 minutes at 50°C, 20°C/minutes to 225°C held for 2 min, 20°C/min to 300°C held for 5 minutes. In total, the run time was 24.5 minutes. The mass spectrometer was operated in full scan mode from 40 to 500 AMU. Mass spectra for recorded peaks were further evaluated using the NIST database (MS search programme Version 2.0, NIST, MSS Ltd., Manchester, England). The analysis of the smoke samples was performed on 5 separate cannabis samples from each of the two batches considered.

2.2.4 Cannabis Smoke - Pulmonary Surfactant Interaction

To elucidate the influence of cannabis smoke on the Curosurf® system under physiologically relevant conditions, the aerosolised material was transferred to the lung biosimulator as previously described [12]. Langmuir isotherms were obtained from a single compression towards the centre of the compartment at a barrier speed of 25cm^2/min. Whilst, Langmuir isocycles were recorded with 14 compression-expansion cycles at a rate of 100cm^2/min. At this stage, initial isocycles (n=4) were used to condition the surfactant monolayer such that the equilibrium position was reached. This strategy provided a much more physiologically relevant demonstration of the influence of cannabis vapour on the simulated pulmonary surfactant monolayer (i.e. as per tidal breathing). All Langmuir isotherm tests were repeated in triplicate, with the same true for Langmuir isocycles. Average data sets are presented within this study including the standard error of the mean. On test completion, the vapour within the lung biosimulator was removed into the fume hood via compressed air.

2.2.5 Langmuir Monolayer Compressibility

Langmuir monolayer compressibility relates to the capacity of the two-dimensional surfactant film to lower the surface tension term at the air-liquid interface with minimal change in surface area [30]. Ideally, pulmonary surfactant should exhibit low compressibility values to enable gaseous exchange to take place over a large surface area [31]. The lower the compressibility term, the more rigid the surfactant film is (i.e. of lower elasticity), with the opposite true [32 & 33]. The parameter is calculated as per Equation 1.

$$\text{Compressibility} = \frac{1}{\text{A}} \text{x} \frac{1}{\text{m}}$$

Equation 1. *Lung Surfactant Compressibility Determination.*

Where A represents the relative surface area and m the slope of the isotherm. Here, 'm' was calculated via 'm = $\frac{y2-y1}{x2-x1}$, over the surface pressure range of 20-35mN/m, whereby 'y' and 'x' values characterise surface pressure and percentage trough area values, respectively [30].

3. Results and Discussion

3.1 Analysis of Cannabis Smoke Extracts

Typical GC-MS data from the cannabis samples are illustrated in Figure 4.

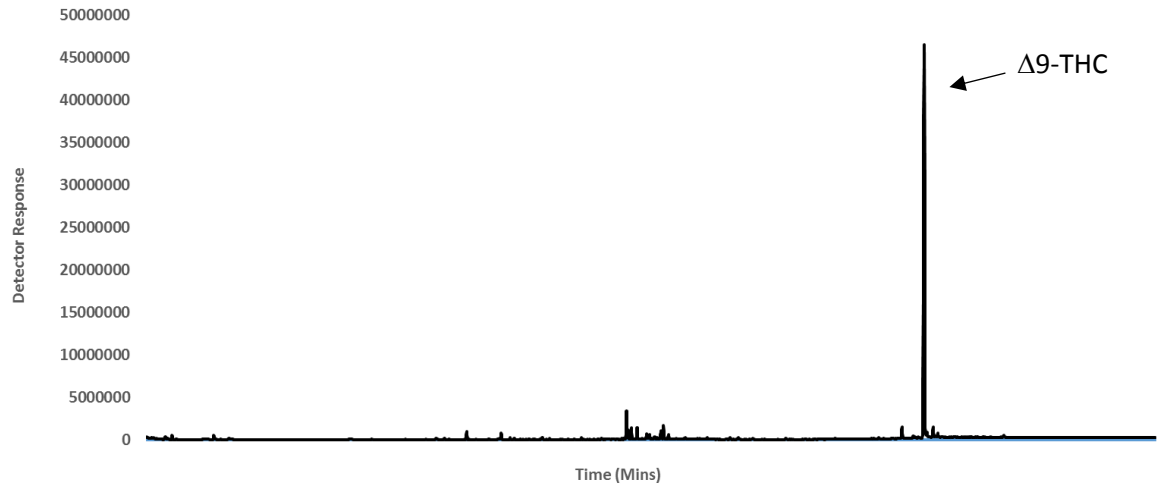

Figure 4. *GC-MS Analysis of Cannabis Smoke Indicating the Major Component ∆9-THC.*

The chromatographic data confirm that the major component of cannabis smoke is ∆9-THC. Therefore, this particular molecule is anticipated to dominate the interaction with the Curosurf® system applied herein.

3.2 Cannabis Quantification

The concentration of ∆9-THC present within the smoke aliquots generated from the two batches of cannabis considered during this work are presented in Table 1.

Cannabis Batch	∆9-THC Concentration (µg/ml)	Percentage ∆9-THC (%)	Percentage RSD (%)
1	480.43	0.96	20
2	550.05	1.10	18

Table 1. *Cannabis concentrations of the cannabis smoke samples from various batches tested.*

The results confirm different levels of ∆9-THC between those batches analysed, with batch 1 containing a lower amount of ∆9-THC. The apparent variability observed within the data set may be ascribed to inherent heterogeneity within the naturally occurring plant material as well as variability in the smoking process. By igniting the plant material, a variety of thermolysis and combustion products are produced, in addition to approximately 30% of the THC being lost due to oxidative degradation [34]. The method of smoke production also influences the concentration of THC in smoke. For example, THC concentration has been shown to increase if the puff frequency is shortened, and the puff volume and length are increased [35].

3.3 Langmuir Pressure – Area Isotherms

Langmuir pressure-area isotherms were obtained for the Curosurf® surfactant system when exposed to cannabis smoke under physiologically relevant conditions; the data sets are presented in Figure 5.

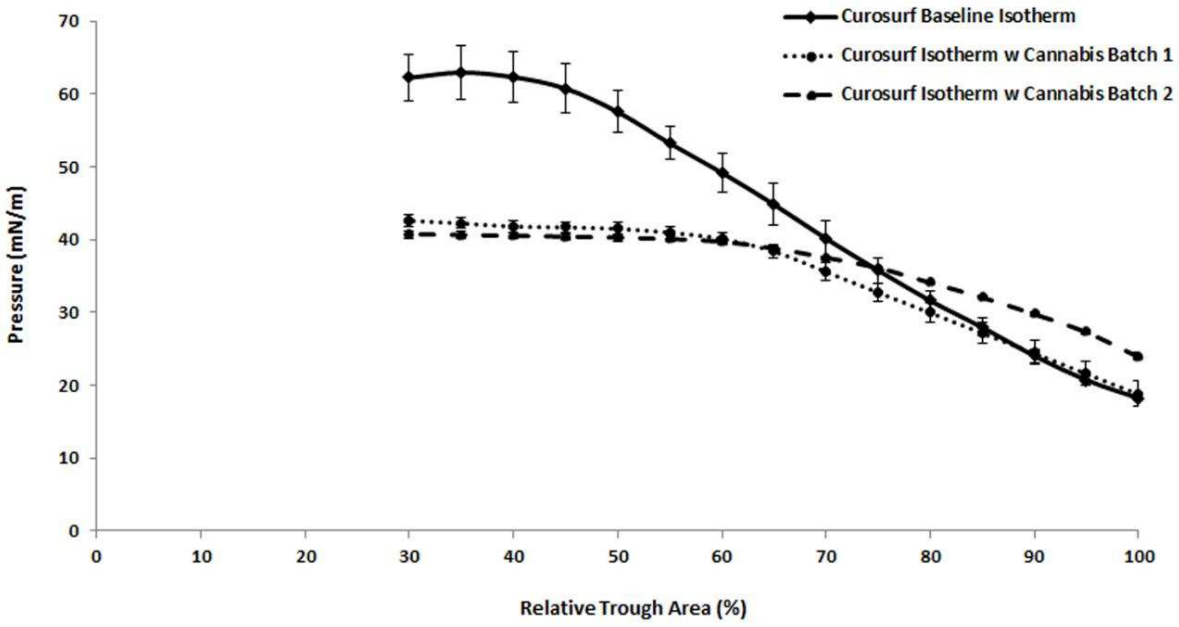

Figure 5. *Langmuir pressure-area isotherms detailing the response of the Curosurf® surfactant system to cannabis smoke addition under physiologically relevant conditions, namely 37°C and elevated relative humidity. Averaged data of 3 replicates presented with standard error of the mean displayed.*

The Curosurf® system experienced two-dimensional phase changes over the course of compression. Deviation in the physical arrangement of the monolayer across the two-dimensional plane (i.e. movement from the gaseous phase through to the solid phase) is confirmed on gradient change from right to left. It is apparent that cannabis smoke caused a detrimental impact upon the performance of Curosurf®. Exposure to 'Batch 1' cannabis smoke caused a statistically significant ($p=0.010$) reduction in surface pressure across all areas when compared to the baseline data. In addition, the maximum surface pressure was reduced from 62mN/m to 43mN/m; signifying a 31% reduction. The clear reduction in surface pressure underscores a reduced ability of the material to attain low surface tension values, which are vital for effective lung function.

A similar effect was noted in the case of 'Batch 2' cannabis smoke. At compression end, exposure to 'Batch 2' cannabis smoke reduced the maximum surface pressure of the Curosurf® product from 62mN/m to 41mN/m, indicative of a 34% decrease. In both cases of cannabis smoke exposure, the Langmuir isotherms demonstrated a condensed nature relative to the baseline as compression proceeded. A statistically significant ($p=0.05$) difference was observed between the influence of 'Batch 1' and 'Batch 2' cannabis smoke starting from a relative trough area of 65%. This variance correlates well with the THC content of each batch as detailed in Table 1. We note that the higher THC concentration produced a greater magnitude of surface pressure reduction.

Consideration was also afforded to the compressibility of the surfactant film under investigation herein. The forward sweep gradient of a Langmuir isotherm / isocycle slope can be used as a marker to assess the compressibility of the two-dimensional film; where the steeper the slope, the harder it is to compress the surfactant monolayer [36]. On inspection of the data presented in Figure 5, it is evident that on exposure to both cannabis smoke batches compression of the Curosurf® monolayer system required less work when compared to the baseline.

In order to quantify the influence of cannabis smoke upon the compressibility of the Curosurf® product, the gradient along the liquid-condensed to liquid-expanded region was considered (i.e. between the surface pressure values of 20 mN/m and 35 mN/m at relative trough areas of 40%, 60% and 80%). The results acquired from this element of the study are presented in Figure 6.

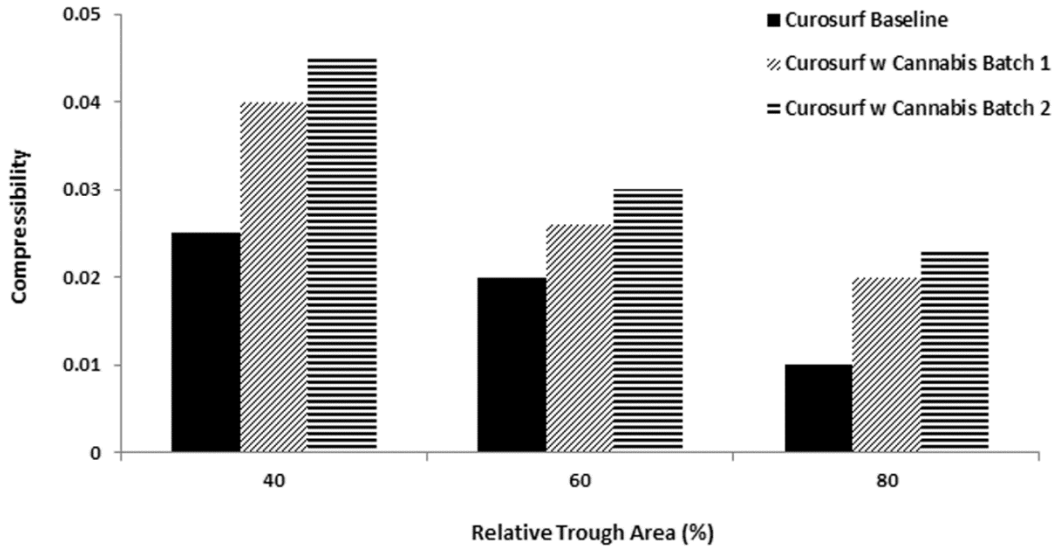

Figure 6. *Compressibility data relating to Curosurf® surfactant monolayers in the presence and absence of cannabis smoke at pre-defined relative trough areas. The delivery of the smoke from both batches increased compressibility in all cases.*

Following exposure to both batches of cannabis smoke, there was an increase in the compressibility term relative to the pristine monolayer. Here, cannabis smoke from 'Batch 2' caused the largest increase in the compressibility term as compared to baseline. The results indicate that contact with the environmental stressor results in a more readily compressible system.

The application of single Langmuir isotherm compressions to probe the performance of a surfactant film within the (deep) lung is not ideal. This is so because the surface active material dispersed across the two-dimensional plane within the laboratory setting is not subject to true hydrodynamics as per the pulmonary space during tidal breathing. Thus, the influence of cannabis smoke upon Curosurf® during single compression studies is not representative of the *in vivo* scenario. Despite this point, information held within such data sets is valuable because it can be utilised to advance our understanding of pulmonary surfactant dynamics, and in particular likely protective mechanisms displayed by the material within the body over time [12].

Importantly, Langmuir isotherm data can be used to provide an insight into the effects of stressors (e.g. cannabis smoke) on exposed, *individual* molecular species especially those molecules in the gaseous phase prior to barrier compression. Here, the delivery of cannabis smoke to the lung biosimulator complimented that of normal use in that the aerosolised material interacted with the model interface via a 'top down' approach, with the relatively exposed monolayer components in the randomly oriented gaseous phase.

3.4 Langmuir Pressure – Area Isocycles

Langmuir pressure-area isocycles were completed for each system under consideration. Here, complementarity with the *in vivo* scenario was achieved and this facilitated a true assessment of the influence of cannabis smoke on Curosurf® performance; the data set acquired from this part of the study is presented in Figure 7.

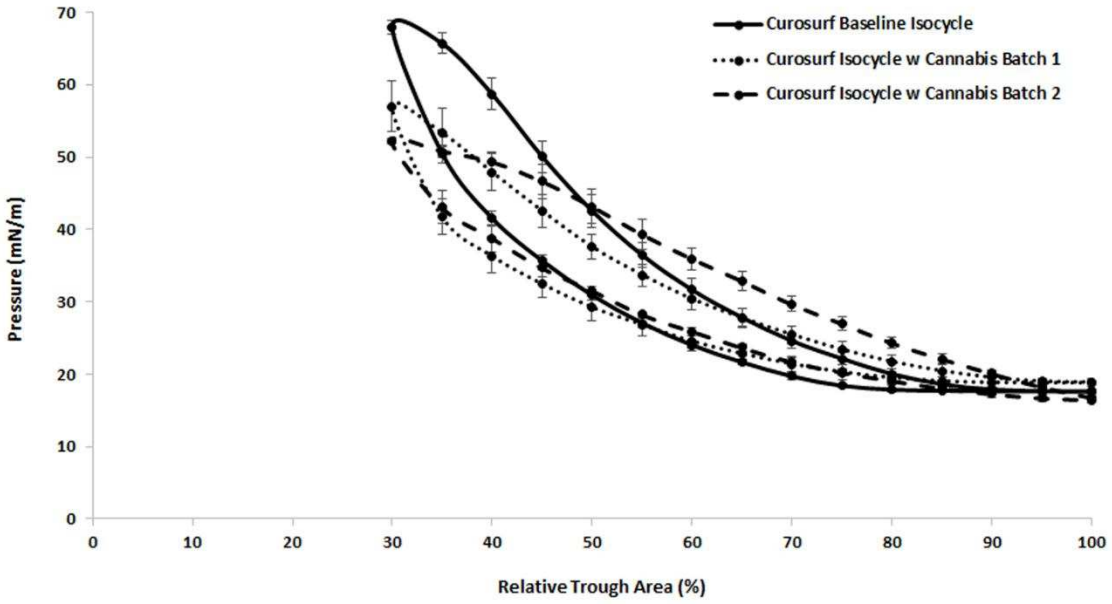

Figure 7. *Langmuir isocycle data relating to the response of Curosurf® to cannabis smoke under physiologically relevant conditions, namely 37°C and elevated relative humidity. Averaged data of 3 replicates presented with standard error of the mean displayed. Where, each replicate consists of 10 compression-expansion cycles at a barrier speed of 100cm^2 / min.*

As previously detailed, compression of the Curosurf® surfactant film towards the centre of the compartment resulted in phase changes within the ensemble. Clear hysteresis in all data sets is apparent on film relaxation towards the start point. This is a well-recognised and natural phenomena that is ascribed to interactions taking place between constituent molecules of the surfactant film. The result confirms that the surface active material is able to respread effectively, even after exposure to cannabis smoke samples. When compared to the pristine system the capacity to lower the surface tension term was reduced following contact with cannabis smoke. The Curosurf® product attained a maximum surface pressure value of 68mN/m (i.e. average value of n=3 repeats), which confirms suitability as an effective lung surfactant replacement preparation [27].

On exposure to 'Batch 1' cannabis smoke, a statistically significant (p=0.039) reduction in surface pressure between relative trough areas 95% and 30% with a maximum surface pressure reduction of 16% (from 68mN/m to 57mN/m) was noted. This highlights the reduced ability of Curosurf® to attain low surface tensions. In addition, on visualisation of the data presented in Figure 7, it is evident that the Langmuir isocycle associated with 'Batch 1' cannabis smoke exposure exhibited an overall condensed character but initially presented as an expanded monolayer during the initial phase of compression. A similar effect on Curosurf® performance was noted following the test with 'Batch 2' cannabis smoke. Again, exposure to 'Batch 2' cannabis smoke caused a statistically significant (p=0.045) reduction in surface pressure between relative trough areas 55% and 30%. Moreover, this batch of cannabis caused a larger decrease in maximum surface pressure relative to 'Batch 1' with a surface pressure reduction of 24% from baseline (i.e. decrease in surface pressure from 68mN/m to 52mN/m). This finding confirms a correlation between the THC levels within the herbal batch and the influence on surfactant surface pressure reduction. The finding was further reinforced by the statistically significant (p=0.024) difference in activity between 'Batch 1' and 'Batch 2' cannabis smoke across all trough areas. Importantly, maximum surface pressure reductions in the case of the Langmuir isocycles were of lower magnitude relative to the Langmuir isotherms. This observation is suggestive of a potential innate 'protective mechanism' attained by the monolayer during the Langmuir isocycle compression / expansion cycles [12]. In a similar manner to the Langmuir isotherm data, gradients of the slopes at surface pressures between 20mN/m and 35mN/m at relative trough areas 40%, 60% and 80% were used to calculate the compressibility term. The results from this arm of the investigation are presented in Figure 8.

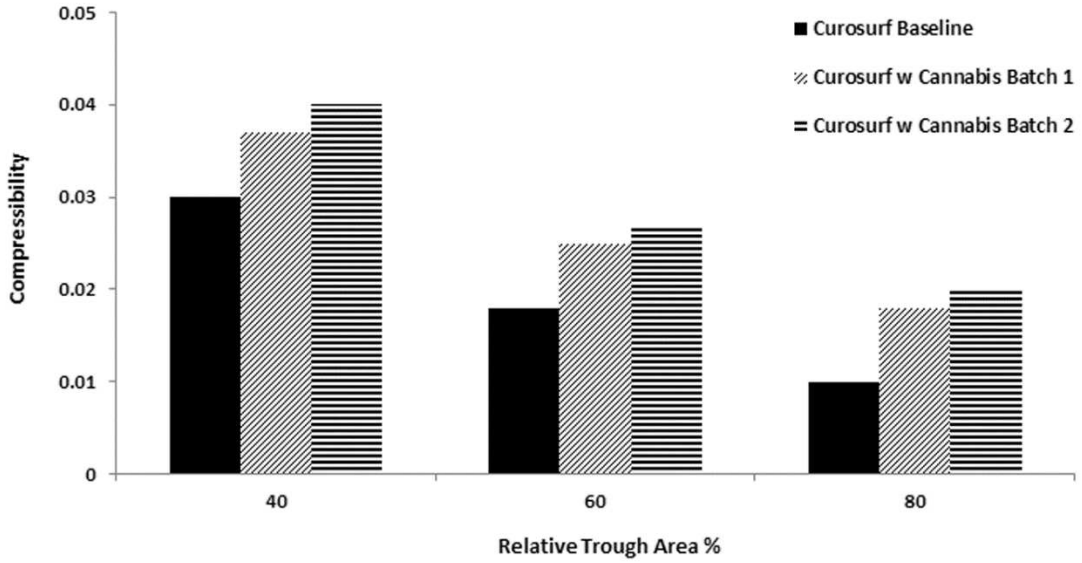

Figure 8. *Compressibility data relating to Curosurf® surfactant monolayers in the presence and absence of the cannabis smoke at pre-defined relative trough areas. The delivery of smoke from both batches increased the compressibility term throughout.*

Exposure to cannabis smoke increased the compressibility term relative to the pristine system. Thus, the monolayer became more compressible and less rigid when challenged with the environmental stressor. The smoke generated from 'Batch 2' cannabis caused the largest increase in the compressibility term at all relative trough areas, which correlated well with the reduction in the surface pressure term (i.e. same trend apparent). Relative to the pristine monolayer, the compressibility values at relative trough areas 40%, 60% and 80% increased by 23%, 39% and 80% upon exposure to 'Batch 1' cannabis smoke and 33%, 50% and 100% for 'Batch 2' cannabis smoke. The relative increases in compressibility observed during the Langmuir isocycles are lower compared with the Langmuir isotherm data. This suggests that compression / expansion cycling conferred some degree of resistance or protection to the monolayer against increases in compressibility (i.e. the negative impact of cannabis smoke was reduced further to monolayer cycling as per the breathing cycle in the (deep) lung).

Clearly, Langmuir isocycle data offers a more representative insight into the effect of cannabis smoke on the human respiratory system as compared to single monolayer compressions. In a similar fashion to the Langmuir isotherm data, the results confirmed that exposure to both batches of cannabis impaired the ability of Curosurf® to reduce the surface tension term and led to an increase in the general compressibility of the surfactant film. Importantly, the reduction in the surface pressure term was much reduced when compared to the Langmuir isotherm data. We suggest that the difference noted between the data sets is due to the synergistic effect of two protective mechanisms; namely the establishment of an equilibrium state and the removal of unstable entities from direct within the monolayer structure. With reference to the first point, during this work we established an equilibrium point (i.e. post 4 initial compression / expansion cycles) such that successive Langmuir isocycles demonstrated limited variance. Attainment of the equilibrium point during monolayer cycling is crucial in order to best represent the *physical arrangement* of the surface active molecules within the (deep) lung. At this point, the molecules within the system would be in a stable conformation and hence align in a manner to support and protect polar functionalities in direct contact with the supporting aqueous subphase beneath. In terms of the second mechanism, we propose that the 'squeeze out' hypotheses results in the removal of unstable or alien (i.e. Δ9-THC) entities from within the monolayer structure and as such the two-dimensional grouping remains stable with time. Further detail regarding this phenomena is outlined below.

The Langmuir isocycle data again presented a correlation between the Δ9-THC concentration of the cannabis batches and their associated detrimental effects upon the surfactant system. It is important to note that although this study has focused on the interaction between the cannabis smoke and Curosurf® monolayers, it has been documented that THC can also alter the synthesis and release of foetal rabbit surfactant related components [37]. Therefore, Δ9-THC may also hold an influence on cellular mechanics that control biosynthetic pathways, which raises the interesting prospect of the potential impact of cannabis smoke not just upon the surfactant system itself but also on those mechanisms that are behind its synthesis and secretion.

3.5 Chemistries of Interaction

The predominantly planar structure of ∆9-THC and its considerable hydrophobic character allows this molecule to readily penetrate into surfactant monolayer structures and create disruptions to impair performance. Although not directly related to the field of Langmuir monolayer technology, this point was highlighted by Cherlet in 2000 who applied nuclear magnetic resonance to confirm within a DPPC bilayer THC assumed a particular orientation that caused membrane perturbations and related disruption [38]. To speculate upon the mechanisms by which THC influenced the Curosurf® system, we will first briefly consider the way in which a surfactant film reduces the surface tension term at the air-liquid interface. Despite being the subject of investigation for in excess of 50 years, the full mechanistic basis by which pulmonary surfactant achieves near zero surface tensions has yet to be fully elucidated. The classical 'squeeze out' model [39] was formulated in an attempt to explain how pulmonary surfactant, which is comprised of only 40% DPPC relative to total phospholipids quantity, can achieve near zero surface tensions at the point of exhalation during the breathing cycle [40]. According to this hypothesis, upon monolayer compression the least stable components within the system are displaced towards the surface associated reservoir. This process may be regarded as an enrichment process for the DPPC molecules located across the two-dimensional plane. These molecules are the most stable and most important surface active agents within the surfactant system in terms of surface tension reduction [41]. Surfactant proteins connect the surfactant molecules to the underlying layers which upon expansion re-spread and allow the return of the expelled molecular species [40].

On delivery of the cannabis smoke to the test zone, the ∆9-THC molecule could interact with the Curosurf® system in various ways. One potential mechanism involves insertion of the planar molecule into the hydrophobic layer, following which there is the development of hydrogen bonds between THC and the phospholipid components (e.g. DPPC). This is made possible by the presence of hydrogen bond donors and acceptors available within all molecular entities involved. As highlighted previously, ∆9-THC contains two important functional groups, namely the phenolic and ether moieties. As the oxygen atom of the ether group is situated in the pyran ring, it is obstructed from participating in hydrogen bonding due to the steric hindrance by the nearby methyl groups. Hence, the only group available to undergo hydrogen bonding interactions is the phenolic group. A destructive interaction within the Curosurf® film would clearly reduce the capacity of the material to perform effectively. This point could manifest by adversely impacting on the enrichment process as noted during monolayer compression (i.e. the 'squeeze out' model).

Furthermore, the retention of fluidiser molecules (i.e. SP-B and SP-C) may explain the apparent increase in monolayer compressibility. As previously outlined, SP-B and SP-C are hydrophobic proteins and are essential in aiding the process of surfactant respread during inhalation. A significant disruption to surfactant structure-function activity (i.e. intercalation of ∆9-THC) could have a negative impact on the ability of the surfactant specific proteins to perform in their role. As an example, during 1990 Hillard and colleagues considered how THC modified the function of membrane associated proteins further to the damaging impact on lipid membrane integrity. Here, the group observed that THC influenced the performance of membrane bound adenylate cyclase enzyme in cardiac cells [42]. Whilst this study is somewhat removed from the current work, scope exists for THC to have altered the function of SP-B and SP-C and as such impair the exchange mechanism between the surfactant associated reservoir and the monolayer in proximity to the supporting aqueous subphase. Notwithstanding this point, THC may clearly interact directly with the surfactant specific proteins via classic molecular interactions (i.e. hydrogen bonding) or via oxidation steps as THC is known to possess oxidising properties [43]. Oxidation of SP-B has been associated with reduction in its function [27].

3.6 Clinical Significance

After tobacco, cannabis is the second most widely smoked substance in the world [44]. The inhalation of cannabis smoke is associated with microscopic injury to the large airways causing inflammation, the production of excess mucus and cough [45]. There is also potential for this herbal material to increase the likelihood of opportunistic respiratory infections [45]. At this present moment in time, information surrounding the impact of cannabis smoke on pulmonary function, and in particular pulmonary surfactant, is limited with data sets being inconclusive [46]. This may be attributed to relatively few scientific studies in the field and a low number of participants within clinical trials [44].

This first in class study considered the direct influence of cannabis smoke on the dynamics of a Curosurf® surfactant monolayer system within an environment reflective of the human lung. Deleterious effects on the pulmonary surfactant replacement product were noted post exposure. When translated to the *in vivo* scenario, the net effect would be a reduction in the support provided by the surface active material to maintain lung, and in particular alveolar stability [31]. Moreover, an increase in the work of breathing would ultimately lead to restricted lung mechanics [47].

Hence, extrapolation of these points can provide a better understanding of how and why various lung pathologies present following the inhalation of cannabis smoke. The experimental data presented herein confirm impairment of lung surfactant function and in real terms this observation can predispose to illnesses such as chronic obstructive pulmonary disease (COPD) [46] and potentially local infections [48]. Indeed, both conditions have been recorded elsewhere in the literature further to the regular inhalation of cannabis smoke [49]. As such, consumers of cannabis likely to develop restrictive lung diseases (e.g. asthma), and experience a range of lung infections (e.g. pneumonia). Overall, it is likely that the regular inhalation of cannabis smoke will result in the individual experiencing increased morbidity and a reduction in quality of life over the longer term.

4. Conclusion

This interdisciplinary study has conclusively demonstrated that cannabis smoke can adversely affect the performance of the clinically relevant pulmonary surfactant replacement product Curosurf®, under conditions as per the (deep) lung. Here, we have presented statistically significant evidence that confirms when a Curosurf® surfactant film is exposed to cannabis smoke it becomes more compressible (i.e. more elastic in nature) and the ability of the material to attain low surface tension values is compromised. In terms of the *in vivo* scenario, the individual is expected to experience an increased work of breathing following the inhalation of this environmental stressor. Moreover, chronic exposure is likely to promote a number of respiratory disorders (i.e. asthma and COPD) and this is ascribed to the negative impact directly upon the pulmonary surfactant function. The authors propose key mechanisms of interaction include: a) insertion of the relatively hydrophobic Δ9-THC molecule into the two-dimensional monolayer structure and subsequent destructive association (i.e. hydrogen bonding) with amphiphilic components plus b) direct interaction between Δ9-THC with SP-B and SP-C molecules to influence their function and cause disturbance across the surfactant film.

Importantly, the lung biosimulator has been successfully applied to model the pulmonary space and assess the impact that environmental stressors may have on respiratory mechanics. This technology platform holds great potential to advance current understanding regarding drug delivery to the lung. The lung biosimulator offers incredible versatility to the operator within the laboratory setting.

Not only is the device capable of quantitatively probing the impact of inhaled toxins on pulmonary function, it can also be utilised to execute scientific investigations ranging from orally inhaled product (OIP) dissolution profiling to drug partitioning studies under biologically relevant conditions. Thus, we believe that this concept displays remarkable promise to play an instrumental role in aiding scientific understanding of how environmental stressors may impinge upon the respiratory system as a whole.

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