The Influence of Cannabis Smoke and Cannabis Vapour on Simulated Lung Surfactant Function under Physiologically Relevant Conditions

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Abstract

The use of cannabis for medicinal / recreational purposes is widespread throughout the world. Smoke inhalation is known to cause airway irritation due to noxious substances (i.e. benzene) within the mix. Thus, advanced vaporisation platforms (e.g. Davinci IQ) have been developed to circumvent negative health implications. Here, we consider the impact that cannabis smoke and cannabis vapour have on simulated lung surfactant performance within a model pulmonary space (i.e. 37°C, elevated humidity and related fluid hydrodynamics). In total, 50mg of herbal material was ignited or placed within a Davinci IQ vaporiser with subsequent activation. The aliquots were collected and then analysed using gas-chromatography – mass spectroscopy for composition and cannabinoid (e.g. Δ9-tetrahydrocannabinol (Δ9-THC)) concentration. The average content within cannabis smoke was 2.84% (0.07%, SD) Δ9-THC, with the same for cannabis vapour being 0.88% (0.14%, SD). Aerosolised samples were transferred to the lung biosimulator. When compared to the pristine Curosurf® system, challenge with cannabis smoke and cannabis vapour reduced the surface pressure term by 26% and 7% and increased film compressibility by 60% and 15% at 80% trough area, respectively. The net effect would be enhanced film elasticity and an increased work of breathing; being more pronounced on cannabis smoke inhalation. The trends noted were ascribed to two factors operating synergistically; namely the amount of Δ9-THC (plus others) within the aerosolised samples and the associated toxicity profile. Further research is required to establish mass-balance effects (i.e. titrated outputs) along with detailed chemical profiling of material generated from the unrelated cannabis activation pathways.

Key words

Cannabis Smoke, Cannabis Vapour, Langmuir monolayers, pulmonary surfactant, lung biosimulator, gas chromatography – mass spectroscopy.

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1. Introduction

The cannabis plant contains numerous chemicals, such as cannabinoids and terpenes [1]. The administration of cannabinoids to the body results in psychoactive effects by way of altering alter neurotransmitter release within the endogenous cannabinoid system (ECS) [2]. The main psychoactive component within the cannabis plant is ∆9-tetrahydrocannabinol (∆9-THC) [3]. It is thought that ∆9-THC acts synergistically with the endogenous cannabinoids (i.e. anandamide and 2-arachidonoyl) by activation of four G-protein coupled receptors within the ECS. Further to receptor (i.e. CB1 and CB2) stimulation, anxiolytic and analgesic properties result [4]. Cannabis is known to cause fatigue, altered sensory perception and contribute to mental health conditions such as schizophrenia [2, 4]. Terpenes, such as linalool and limonene, are aromatic molecules responsible for the distinctive organoleptic properties of cannabis [5].

Cannabis is the most extensively cultivated, trafficked and recreationally used illicit drug in the world [6]. The respiratory system is frequently used as a means to deliver recreational drugs (e.g. cannabis) to the body in order to derive the effects as outlined above. Drug substances delivered to the lung are able to diffuse into the systemic circulation further to contact with the pulmonary air-liquid interface. To date, limited consideration has been given to the impact of cannabis smoke and cannabis vapour may have on the human airway, and in particular pulmonary surfactant [4]. Given the widespread use, it is important to understand the effects of cannabis and its related compounds on the body (e.g. the lungs) so we can better understand the impact of these chemicals on public health as a whole.

The inhalation of smoke from various sources (i.e. the tobacco or the cannabis plant) is known to be deleterious to the body [7]. This is so as the combustion process liberates noxious substances including benzene, carbon monoxide and tar [8, 9]. Pulmonary irritation and depleted surfactant levels result further to oxidative stress, which leads to deviation in respiratory mechanics from the norm [10]. Within recent years, advanced vaporisation platforms (e.g. the Davinci IQ) have emerged within the marketplace. This technology is viewed as an alternative to traditional smoking that negates the harmful effects caused by smoking plant-based material. The process of drug vaporisation, or ‘vaping’ as it is frequently referred to, involves the production of a drug-containing aerosol further to the heating of dried herbal material via contact with a heating element [11].

Vaporisers are considered to offer a 'clean' alternative to smoking, with levels of particulates and volatile organic compounds no higher than daily background levels [8, 12]. Indeed, during 2017 Shahab and co-workers highlighted the fact that former smokers using vaporised nicotine-replacement therapy had lower concentrations of harmful metabolites associated with cigarette smoke consumption, but similar concentrations of nicotine when compared to current smokers [11].

The lung biosimulator [13], a recent development in the field of Langmuir monolayer technology, can be applied within the laboratory setting to investigate the effects of cannabis smoke and cannabis vapour on pulmonary surfactant monolayers as per the (deep) lung [3]. Langmuir pressure-area isocycles arising from the advanced biomedical strategy offer an indication as to the dynamics (i.e. compression / expansion functionality) of surfactant material during the process of tidal breathing when exposed to environmental stressors [14]. Naturally, comparisons can be drawn between baseline data when challenged with environmental stressors. Here, the pulmonary surfactant replacement product called Curosurf® (i.e. that prescribed to manage respiratory distress syndrome) was applied as a model alveolar air-liquid interface [15]. The product is a mixture of dipalmitoylphosphatidylcholine (DPPC) plus surfactant proteins B and C [16]. On delivery to the respiratory system, the material forms a thin solid film to reduce the surface tension term and promote tidal breathing [17].

The aim of this research is to determine the influence cannabis smoke and cannabis vapour hold on simulated pulmonary surfactant monolayers within a model lung environment. During this piece, consideration will be given to the chromatographic analysis of both cannabis smoke and cannabis vapour aliquots, mass-balance dose comparability, chemistries of interaction along with the relevance of this work to public health.

2. Materials and Methods

2.1 Materials

The commercially available lung surfactant preparation Curosurf® (Chiesi Ltd, Italy. Lot: 1065833) was applied herein to represent the alveolar air-liquid interface. As previously detailed, a dilution step was applied prior to Curosurf® use [3]. In summary, a buffer solution comprising NaCl (150mM), $CaCl_2$ (2mM) and $NaHCO_3$ (0.2mM) at pH 7 was used to dilute the surfactant product from 80mg/ml phospholipid concentration to 1mg/ml phospholipid concentration [17]. Once this dilution stage was complete, a suitable volume of the spreading solution was placed across the ultrapure water subphase held within the Langmuir trough to increase the surface pressure from 0mN/m to approximately 16mN/m [18]. Two batches of cannabis were applied during this work (i.e. Batch A and Batch B).

The herbal material was used as supplied and either pyrolysed as previously outlined to generate smoke aliquots [3] or activated within an advanced vaporiser platform (i.e. Davinci IQ) to generate vapour aliquots. In both cases, the cannabis was acquired from Merseyside Police under a Home Office Research Licence. 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 used as the solvent to facilitate chromatographic analysis. Ultrapure water (Purite, UK), demonstrating a resistivity of 18 MΩ.cm and pH 7, was used as the cleaning solvent 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 [13]. Surfactant free tissues (Kimtech Science, Kimberley-Clark Professional, 75512, UK) were soaked in chloroform and applied to clean the glassware and contacting surfaces. Cleanliness was assured within the Langmuir trough (Model 102M, Nima Technology, UK) by attaining a surface pressure value of ≤0.4mN/m on full barrier compression. A Hamilton microsyringe was applied to deliver the diluted Curosurf® to the surface of the ultrapure water subphase.

Here, sufficient amphiphilic material was delivered to reach a starting pressure of 16mN/m and a wait period of 10 minutes then allowed to enable material spread over the 70cm^2 aqueous surface. In order to acquire Langmuir isocycle data, the trough barriers were programmed to move inward / outward relative to the centre of the compartment at 100cm^2/min. Langmuir surface pressure vs. percentage trough area at 37°C and elevated humidity (e.g. 85% RH) were obtained via a Wilhelmy plate positioned in the middle to the Langmuir trough.

2.2.2 Cannabis Smoke / Vapour Generation

At the outset, all glassware was thoroughly cleaned and subsequently connected together using Tygon tubing to form an airtight system, as illustrated in Figure 1. The generation of cannabis smoke and cannabis vapour was undertaken in a fume hood to restrict unwanted release of aerosolised material within the laboratory space. Here, the starting material was either ignited as previously described [3] or placed within the Davinci IQ platform and activated at full battery power under the manufacturer's factory default conditions (i.e. maximum temperature of 221°C).

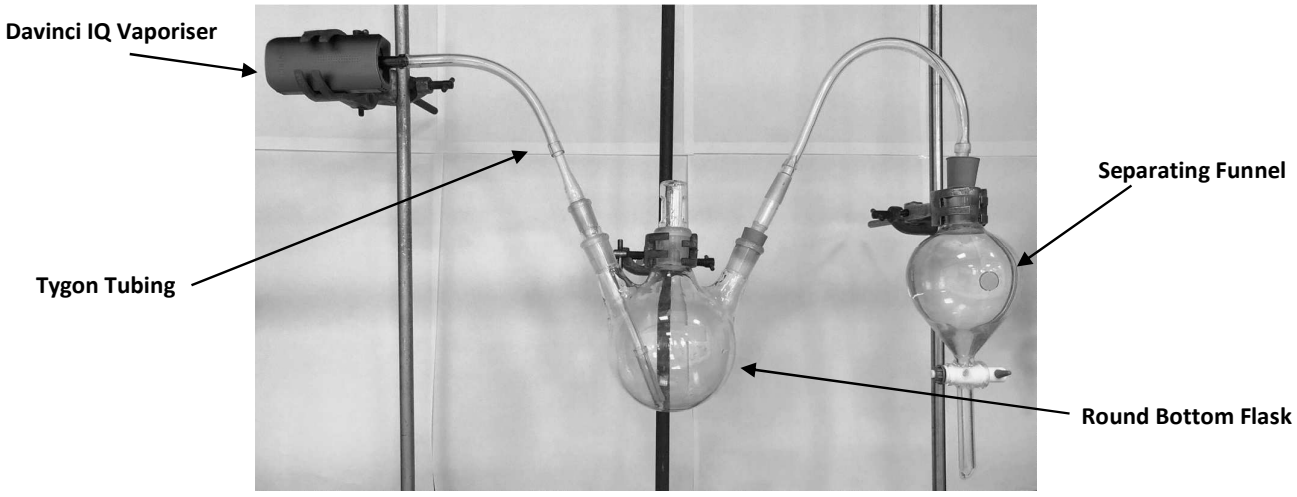

Figure 1. *The experimental arrangement applied to collect cannabis vapour aliquots, showing the Davinci IQ platform. This advanced vaporisation device was detached and replaced with a glass pipe plus perforated foil support during the pyrolysis experiments, as previously detailed [3].*

A total of 50mg of ground cannabis [9] was pyrolysed by igniting with a long-necked electronic lighter. With regards to the vaporisation process, a mass of 50mg cannabis was placed inside the Davinci IQ platform and the device then activated at maximum battery charge and under the manufacturer's baseline settings. Here, the intention was to achieve a consistent starting mass (i.e. 50mg), prior to material activation. On activation of both systems, a volume of 50ml of water was pulled through the 250ml separating funnel to create a vacuum. Related smoke / vapour was collected in the 500ml round bottom flask and allowed to settle for 30 seconds. A further 50ml of water was then pulled through and this process was repeated until a total volume of 250ml of water was used (i.e. 5 puffs). The tubing connecting the round bottom flask was removed and stoppers inserted immediately to ensure no loss of cannabis smoke / vapour. At that stage the cannabis smoke / vapour sample could be delivered directly to the lung biosimulator. For GC-MS analysis, the smoke / vapour was passed through a volume of 10ml of analytical grade methanol to solubilise the smoke aliquot.

2.2.3 Chromatographic Analysis of Cannabis Smoke / Vapour

The analysis of the smoke / vapour samples and THC standards (10 -100µg/ml range) was performed in triplicate using an Agilent 6980GC with 5975MS detection. Hexadecane was added as an internal standard. The column was an Agilent J&W HP5-MSUI with the dimensions of 30m x 0.250mm x 0.25mm run in splitless mode with an 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, and single ion monitoring mode (CBD at 17.95 - 18.20 mins; 231 m/z. THC at 18.2 - 18.6 mins; 314, 299 and 231 m/z. CBN at 18.6 - 23.5 mins; 295 and 310 m/z). Mass spectra for recorded peaks were further evaluated using the NIST database (MS search programme Version 2.0, NIST, MSS Ltd., Manchester, England).

2.2.4 Cannabis Smoke / Vapour - Pulmonary Surfactant Interaction

To determine the impact cannabis smoke and cannabis vapour had on the simulated pulmonary surfactant monolayer system under *in vivo* conditions, the aerosolised material(s) was transferred to the lung biosimulator via compressed air along Tygon tubing. A period of 10 minutes was allowed for related interaction. Langmuir isocycle data was 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 to facilitate a more representative demonstration of the interacting chemistries anticipated during tidal breathing. All data was obtained in triplicate, which enabled the generation of average data sets inclusive of the standard error of the mean.

2.2.5 Langmuir Monolayer Compressibility

The compressibility of a Langmuir monolayer concerns the ability of the thin organic film to reduce the alveolar surface tension with negligible change in surface area [19]. Under ideal circumstances, lung surfactant should exhibit low compressibility values; this allows gaseous over a large surface area [20]. The lower the compressibility of a surfactant film then the more rigid the surfactant film is [21]. Langmuir monolayer compressibility may be determined as outlined in Equation 1.

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

Equation 1. *Langmuir Monolayer Compressibility Determination.*

Where A represents the relative surface area and m the slope of the isotherm. Here, 'm' was calculated via 'm = $\frac{y2-y1}{x2-x1}$' across the percentage trough area values of 60%, 70% and 80%. The related 'y' and 'x' values concern the surface pressure and percentage trough area values, respectively [19].

2.2.6 Statistical Analysis

A one-way analysis of variance (ANOVA) was conducted using the statistics package Minitab (v18.1) in order to determine statistical significance within the data sets. A separate ANOVA was performed on a previously identified range of interest (30-55%) [3], with a Tukey's follow-up test. This latter approach was an appropriate follow up given the unpaired nature of the data and its comparison of each group with every other.

3. Results and Discussion

3.1 Analysis of Cannabis Smoke Extracts

Typical GC-MS data from the cannabis smoke and vapour samples are illustrated in Figures 2 and 3, respectively.

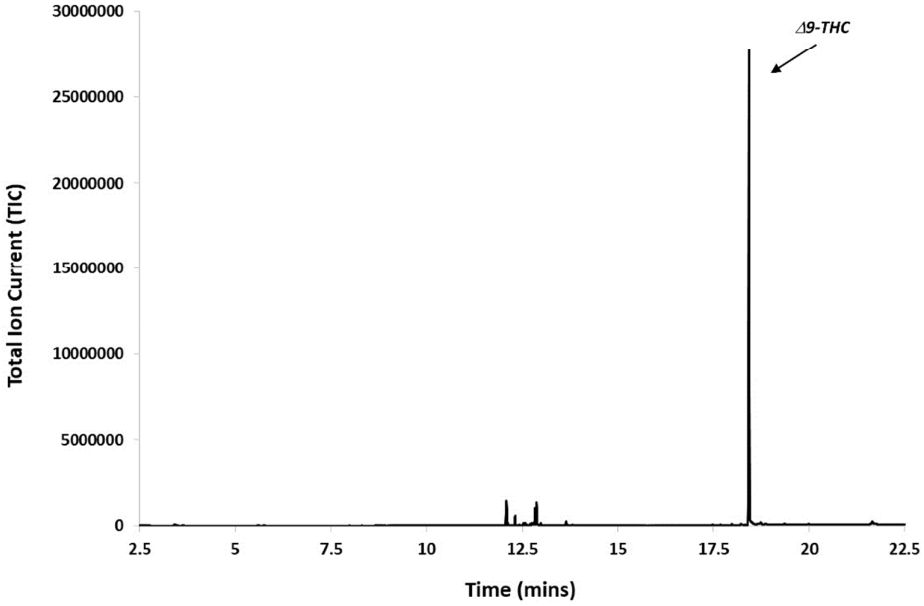

Figure 2. *GC-MS Analysis of Cannabis Smoke Indicating the Major Component ∆9-THC.*

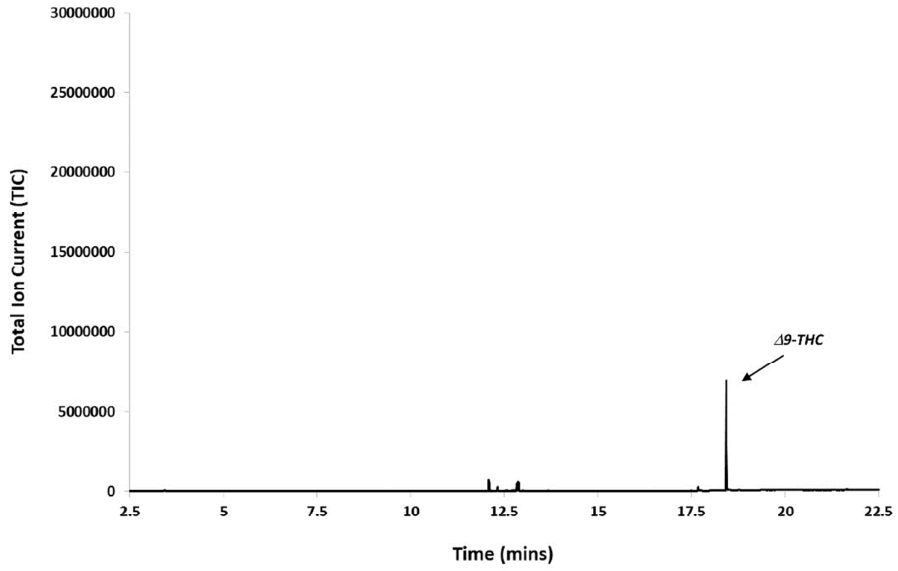

Figure 3. *GC-MS Analysis of Cannabis Vapour Indicating the Major Component ∆9-THC.*

The chromatographic data confirms that the major component of aerosolised cannabis aliquots is Δ9-THC at 18.46 minutes; this is particularly notable in the case of the smoke sample. This molecule will thus dominate the interaction with the Curosurf® system applied herein. The components detected at approximately 12 minutes into the run are commonly occurring terpenes and include for example β-caryophyllene, α-humulene and α-bulnesene. Such terpenes are commonly detected in Cannabis sativa samples as they are synthesised in the same glandular trichomes as cannabinoids [22]. The level of terpene molecules detected was lower in the case of the vaporisation route.

3.2 Cannabis Quantification

Concentrations of the predominant cannabinoid, ∆9-THC, within the cannabis smoke and vapour aliquots are presented in Table 1.

Sample	Replicate	Concentration (mg/ml)	∆9-THC Content (%)	Average Content (%)	Standard Deviation (%)
	1	140.42	2.81		
Cannabis Smoke	2	146.18	2.92	**2.84**	0.07
	3	140.21	2.80		
	1	38.43	0.77		
Cannabis Vapour	2	51.77	1.04	**0.88**	0.14
	3	41.16	0.82		

Table 1. *Concentrations of* ∆9-THC *within cannabis smoke / vapour aliquots*

The data presented in Table 1 indicate that the mode of cannabis activation results in varying amounts of ∆9-THC available for interaction with the Curosurf® system prepared within the lung biosimulator; or in real terms, the (deep) lung. Pyrolysis of the herbal material resulted in an average content of 2.84% ∆9-THC within the aerosolised aliquots collected. However, there was a three-fold reduction in this level was noted with regards to the vaporisation route (i.e. via use of the Davinci IQ platform). Importantly, the variability within the data set is minimal and this we attribute to the execution of the puffing regimen employed herein along with the experimental arrangement (i.e. the use of inert Tygon tubing). Greater variability was noted in the case of the cannabis vaporisation route, and this will be discussed at later juncture in this piece.

3.3 Langmuir Pressure – Area Isocycles

Langmuir pressure-area isocycles were obtained for the pristine Curosurf® system plus the same when exposed to cannabis smoke and vapour aliquots. The data sets for this element of the study are presented in Figure 4.

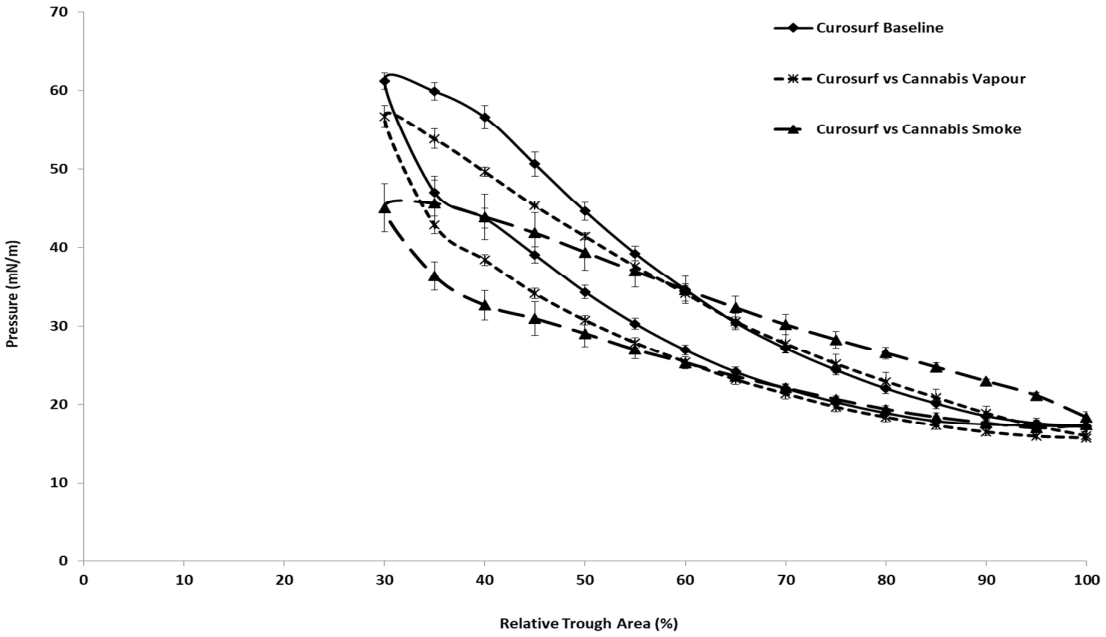

Figure 4. *Langmuir pressure-area isocycle data concerning the exposure of Curosurf® to cannabis smoke and cannabis vapour aliquots under physiologically relevant conditions. Averaged data of 3 replicates presented with standard error of the mean displayed. Where, each replicate consists of 10 compression-expansion cycles at a barrier speed of 100cm^2 / min.*

The data presented in Figure 4 confirm that further to exposure to cannabis smoke and cannabis vapour, the maximum surface pressure for the Curosurf® system decreased. Thus, the ability to reduce the surface tension term at the air-liquid interface was impaired. With regard to the influence of cannabis vapour on the system, the maximum surface pressure for Curosurf® reduced from 61mN/m to 57mN/m. Whilst a clear decline in the value was apparent, and confirmed by the absence of error bar overlap, the difference was found not to statistically significant (i.e. P=0.23, 95% CI). However, on consideration of the influence of cannabis smoke on the maximum surface pressure term for Curosurf® it is evident that exposure caused a reduction to 45mN/m and this was deemed statistically significant (i.e. P<0.01, 95% CI). In order to determine the compressibility term

at pre-defined relative trough areas (i.e. 60%, 70% and 80%) Equation 1 was employed. The output from this element of the study is presented in Figure 5.

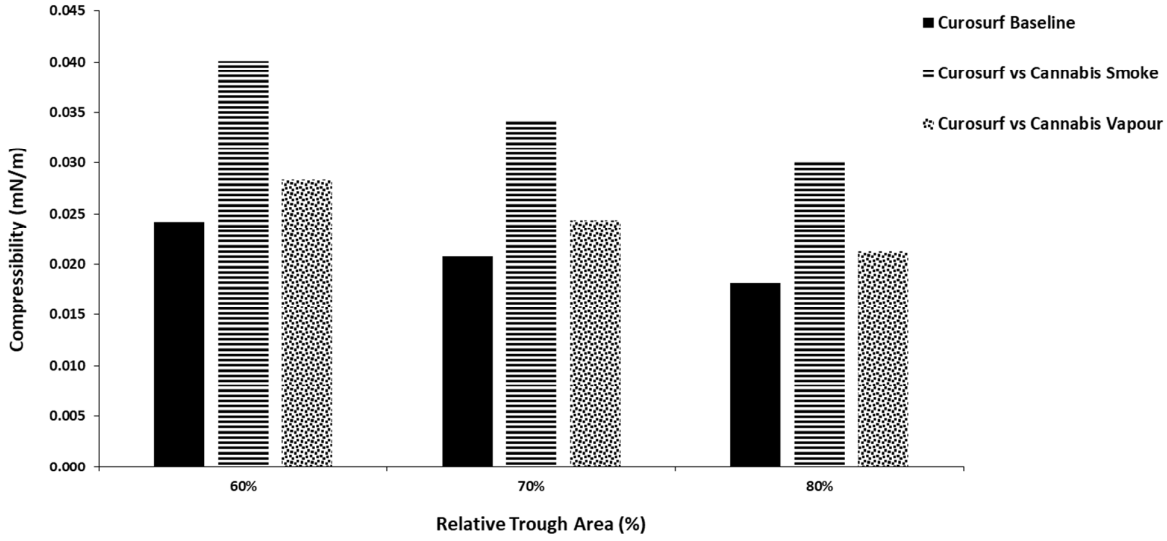

Figure 5. *Compressibility data relating to Curosurf® surfactant monolayers in the presence of cannabis smoke and cannabis vapour at pre-defined relative trough areas. The delivery of cannabis smoke to the test zone increased the compressibility term to the greatest degree.*

The data presented in Figure 5 indicate that exposure to cannabis smoke and vapour aliquots increased the compressibility term, relative to the baseline, throughout. The increase in the compressibility term suggests that the Curosurf® monolayer became more elastic and less rigid when challenged with the aerosolised aliquots. The change in compressibility would suggest that after exposure to cannabis smoke / vapour, the pulmonary surfactant monolayer arrangement was less favourable to support gaseous exchange between the body and the atmosphere [20]. Importantly, if such exchange is affected *in vivo* (e.g. in the case of interstitial lung disease) then the exchange of respiratory gases across the alveolar-capillary barrier would be impaired. This would ultimately result in the lungs not meeting the demands of the body [20, 23].

3.4 Considerations

The results obtained from this study confirm that exposure to cannabis smoke and cannabis vapour was detrimental to the overall performance of the Curosurf® surfactant film. The negative impact was greatest in the case of the cannabis smoke aliquots. Detail regarding the mechanisms of interaction between ∆9-THC and Curosurf® monolayers has been provided elsewhere [3], hence discussion will be limited here. Of greater importance, we believe, are the reasons as to why the trends in our data present. Further to reflection on the experimental procedure and resultant data series, we are mindful that the trend noted could be due to two aspects, namely the differences in ∆9-THC concentrations within the aerosolised aliquots delivered to the lung biosimulator and the toxicity profile associated with each aliquot type. Here, we provide comment on both factors in an attempt to inform on future work in this under-researched and pressing biomedical field (i.e. with the increasing interest in the administration of cannabis for medicinal purposes).

3.4.1 Cannabis Pyrolysis vs Cannabis Vaporisation

Perhaps the most notable point to discuss involving this work is the method used to obtain cannabis smoke and cannabis vapour aliquots for delivery to the lung biosimulator. Here, we utilised 50mg of herbal material for both the pyrolysis route and the vaporisation route (i.e. to achieve a mass balance equivalence) [9]. However, although the same quantity of cannabis was used at the outset, the resultant levels of ∆9-THC within the aerosolised samples differed considerably. In the case of the pyrolysis route, all of the herbal material would have activated and thus been available for interaction with the Curosurf® monolayer held within the lung biosimulator. However, with vaporisation in mind only a fraction of the total mass of cannabis placed into the holding chamber was activated at 221°C. It has previously been reported that the vaporisation of herbal material can lead to variability in drug output during practical use [24]. In the case of our work presented here, this point is reflected in the standard deviation data presented in Table 1 where the variance between activation routes doubles in the case of material vaporisation. There are numerous factors that can influence the vaporisation process; for example, the properties of the heating chamber (i.e. the dimensions and composite material), the location of the herbal material within the chamber if not packed tightly plus the physiochemical properties of the starting material itself [8].

Moreover, user attributes can effect drug release profiles from advanced vaporisation platforms [25]. During this work, we employed an exacting puff regimen to ensure the data were as consistent as possible. However, in reality, the user will exhibit variable puff routines and indeed such puff routines will vary between individuals. Typically, an individual using cannabis for recreational purposes would employ a larger mass within the vaporiser (i.e. pack it full). Furthermore, they would have greater exposure to cannabis than the four 'puffs' method used in this experiment. However, we wanted to maintain a scientific approach in the work and use an equivalent mass for this first-in-class, comparative study. Thus, standardisation during vaporiser use is a major consideration with accurate dose titration and release of a desired / consistent dose of cannabinoids being a significant challenge [24]. This point itself may adversely impede the widespread uptake within the medical community as a whole [9]. We suggest, therefore, that further work should consider the exact mass of herbal cannabis within the vaporiser platform that would reliably generate the same cannabinoid (i.e. Δ9-THC) output as per complete pyrolysis; that is to say a titration-based study for drug release equivalence. This could be achieved, given an appropriate timeframe, by varying the amount of cannabis dry herbal material placed into the vaporiser and analysing the associated output to find the mass that would produce an equivalent amount of the cannabinoid (plus others) as per cannabis smoke.

The chemical composition within the cannabis smoke and cannabis vapour aliquots is another important point for consideration when rationalising the experimental results presented herein. It is widely documented that the vaporisation and subsequent inhalation of drug substance(s) exposes the individual to fewer harmful toxins [7, 25, 26]. Here, we believe that the vaporisation of cannabis and subsequent introduction to the lung biosimulator would have had less of a detrimental effect on the Curosurf® film because the herbal material was heated to the point where cannabinoids were released with no material combustion [7, 27]. The GC-MS data presented within this piece confirm lower concentrations of terpene molecules as compared to full pyrolysis. Indeed, current evidence is supportive of the fact that vaporisation is a safer method of drug administration to the body given the lower levels of polycyclic aromatic hydrocarbons (PAHs), benzene, toluene, naphthalene, and carbon monoxide produced as compared to smoke [7, 28]. Developing this point further, PAHs can induce a range of cytochrome P450 enzymes within the body and thus lead to various drug interactions with medication concomitantly administered. Should the PAH levels be reduced (i.e. via application of vaporisation technology) then it is likely that drug interactions within the body would be minimised.

Interestingly, a number of composite particles within cigarette smoke (e.g. sterols and fatty acids) can interact with surfactant monolayers during the compression phase [29]. Although this study was based upon cannabis smoke and not cigarette smoke, dried cannabis is known to contain many similar constituents capable of disrupting surfactant monolayers such as fatty acids, phenols and hydrocarbons [30]. Cannabis vapour and smoke contains the lipophilic ∆9-THC molecule that is capable of disrupting a surfactant monolayer. This is so as these molecules can insert themselves directly into the monolayer, between surfactant molecules, and interrupt monolayer function; particularly in the solid phase during compression [3, 31]. This would result in a less rigid surface film with increased elasticity, which was noted herein.

3.4.2 Study Relevance – Public Health Aspects

Given the widespread use of cannabis, it is important to fully understand the effects that this herbal material and related compounds have on the body (i.e. the respiratory system) post administration. Cannabis smoke is known to cause lung irritation, frequently resulting in similar airway problems as per cigarette smoke [8, 32]. Signs associated with cannabis smoke inhalation include persistent cough, a high risk of pulmonary infections and excess phlegm production (i.e. respiratory issues) [25, 32]. Pulmonary irritants also result in inflammation, mucosal oedema and protein accumulation within the lung. The net effect is impaired pulmonary integrity [29]. Pulmonary surfactant is important in preventing alveolar collapse, airway stenosis and reduced expiratory flow. As such, deviation in lung surfactant structure-function activity is undesirable for the individual. Although smoking via combustion is a cheaper and easier method of cannabis delivery, vaporisation may offer a promising and safer alternative to the inhalation of combustion products whilst retaining the outcome(s) associated with smoke inhalation [28].

4. Conclusion

This study demonstrates that cannabis smoke has a greater detrimental effect on the surface tension lowering ability and compressibility of pulmonary surfactant monolayers as compared to cannabis vapour. Consequently, further to the inhalation of cannabis smoke the work of breathing for the individual would increase. Chronic exposure to cannabis smoke can result in debilitating respiratory disease (e.g. asthma and obstructive pulmonary function). The vaporisation of cannabis may have a harm reduction effect over the longer term due to reduced levels or the absence of toxic by-products, hence the mechanism holds promise. Additional work is required to establish mass balance effects (i.e. titrated outputs) during cannabis pyrolysis and cannabis vaporisation.

5. Acknowledgements

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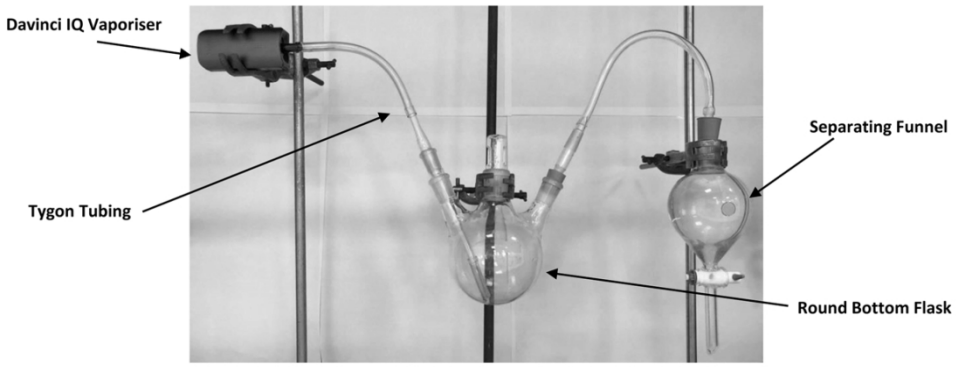

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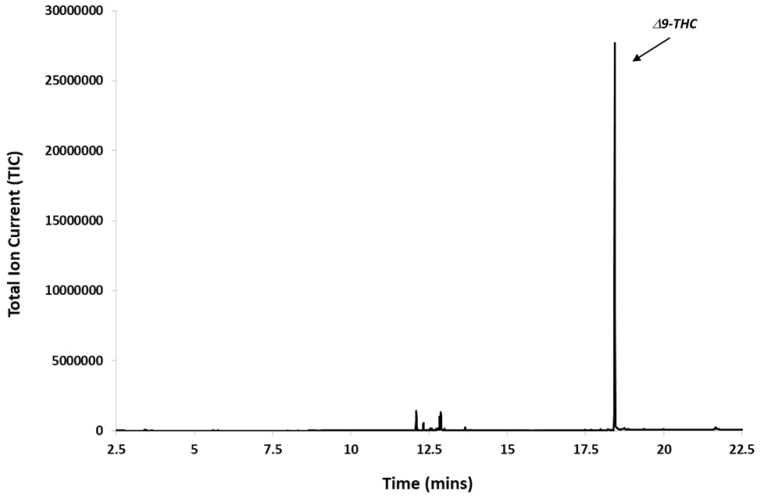

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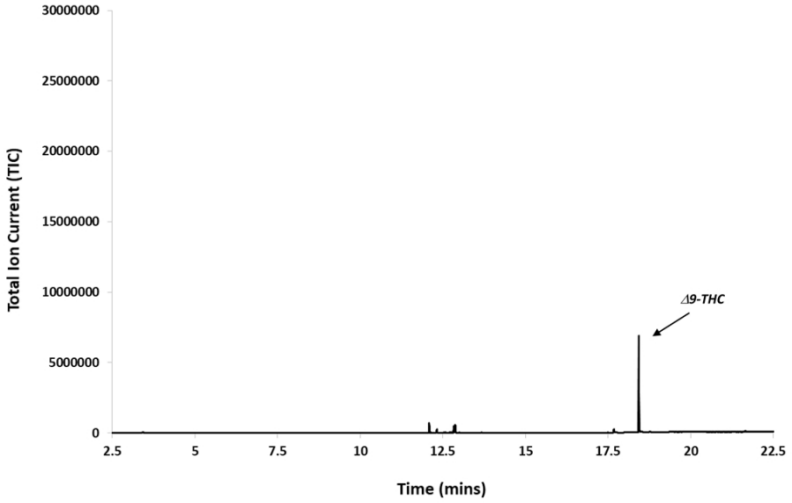

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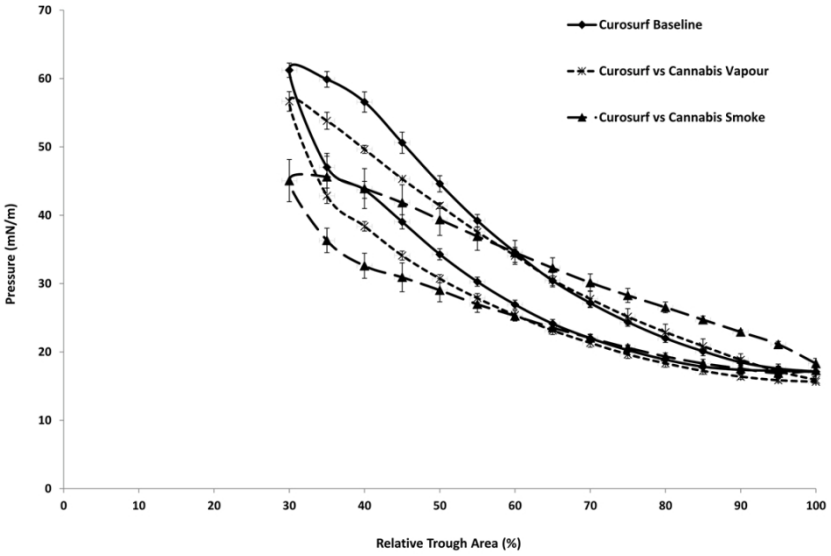

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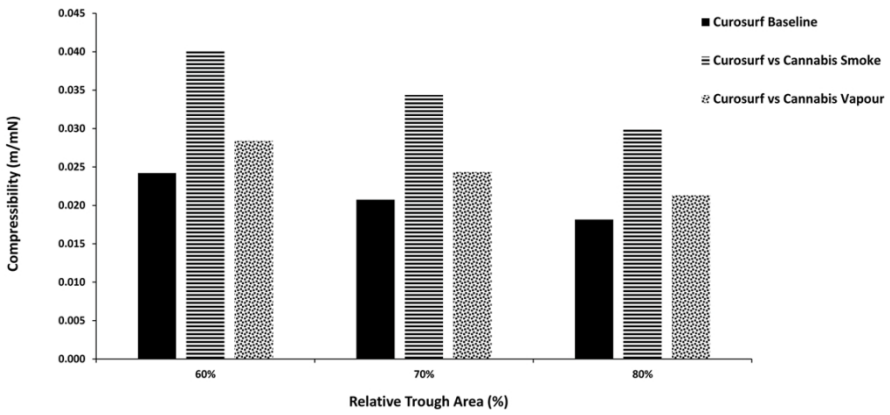

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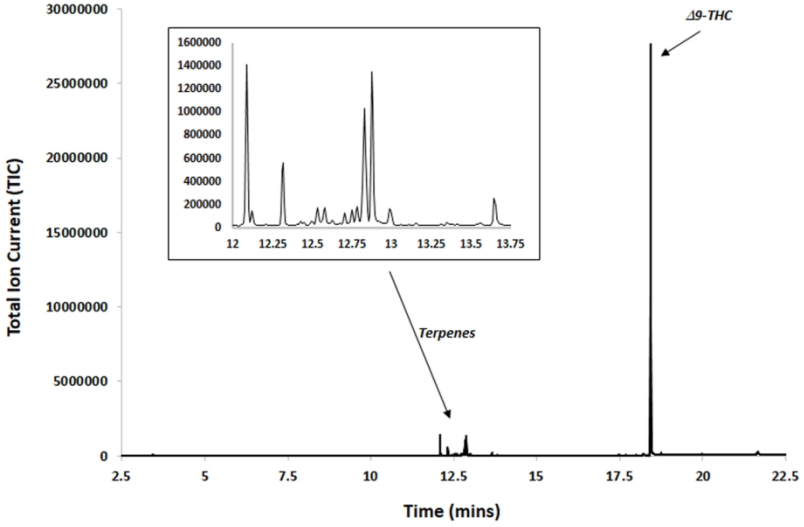
Sample	Replicate	Concentration (mg/ml)	Δ9-THC Content (%)	Average Content (%)	Standard Deviation (%)
	1	140.42	2.81		
Cannabis Smoke	2	146.18	2.92	**2.84**	0.07
	3	140.21	2.80		
	1	38.43	0.77		
Cannabis Vapour	2	51.77	1.04	**0.88**	0.14
	3	41.16	0.82		

338x190mm (96 x 96 DPI)

Common terpenoids identified from the cannabis samples include β-Caryophyllene, the most common sesquiterpenoid in cannabis and caryophyllene oxide, the component responsible for cannabis identification by drug-sniffing dogs.



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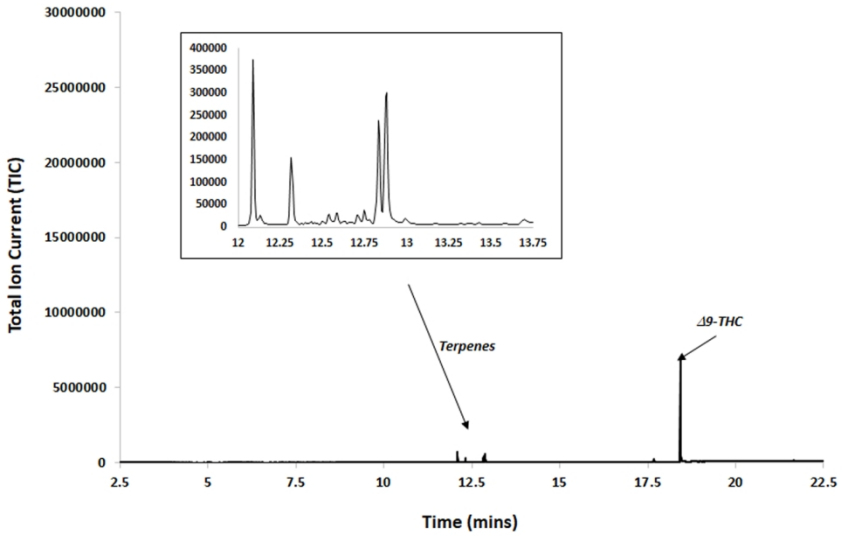

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