AXISYMMETRIC DROP SHAPE ANALYSIS (ADSA)
AND LUNG SURFACTANT

by

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Graduate Department of Mechanical and Industrial Engineering
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Abstract

The objective of this thesis was to further develop a methodology for surface tension measurement called Axisymmetric Drop Shape Analysis (ADSA) and to adapt it to studies of lung surfactants, i.e. the material that coats and facilitates the functioning of the lungs of all mammals. The key property of a functioning lung surfactant is its surface tension, which can reach extremely low values below 1 mJ/m². Such values are difficult to measure; but a certain configuration of ADSA, using a constrained sessile drop (ADSA–CSD), is capable of performing such measurements.

Clinically, lung surfactant films can be altered from both sides, i.e. from the airspace as well as from the bulk liquid phase that carries the film. Therefore, being able to access the interface from both sides is important. Here, ADSA–CSD was redesigned to be used as a micro film balance allowing access to the interface from both gas- and liquid-side. This allows deposition from the gas side as well as complete exchange of the bulk liquid phase. The new design was used to study lung surfactant inhibition and inhibition reversal.

A dynamic compression-relaxation model (CRM) was developed to describe the mechanical properties of lung surfactant films by investigating the response of surface tension to changes in surface area. The model evaluates the quality of lung surfactant preparations – beyond the minimum surface tension value – and calculates the film properties, i.e. elasticity, adsorption and relaxation, independent of the compression protocol.
The accuracy of the surface tension measurement can depend on drop size. A detailed analysis of drop shapes and accuracy of measured surface tension values was performed using a shape parameter concept. Based on this analysis, the design of ADSA–CSD was optimized to facilitate more accurate measurements. The validity analysis was further extended to the more conventional pendant drop setup (ADSA–PD) in which accuracy near \( \pm 0.01 \text{ mJ/m}^2 \) is now possible.

An overall upgrade of both hardware and software of ADSA–CSD, together with extensive numerical work, is described and applied to facilitate a more efficient operation. Finally, it is noted that the ADSA–CSD setup developed here can be used for a wide range of colloid and surface chemical applications.
“I can do all things through Christ who strengthens me.”

(Philippians 4:13)

To my Lord, God and Savior JESUS CHRIST.
To Marie, my parents and my siblings.
To my beloved homeland, Egypt.
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“Trust in the LORD with all your heart, and lean not on your own understanding; in all your ways acknowledge Him, and He shall direct your paths.” (Proverbs 3:5-6).

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Chapter 1

Introduction

1.1 Overview

The significance of interfacial and capillarity phenomena in surface science has been increasingly recognized in recent years. These phenomena occur whenever a liquid is in contact with another fluid or solid. Common examples are menisci, liquid drops formed in either air or another liquid, and thin films such as soap bubbles. Surface phenomena play an important role in many engineering applications and technologies including biotechnology, surface engineering, and micro/nanotechnology.

In the present context, the goal is to adapt a methodology for surface tension measurement called Axisymmetric Drop Shape Analysis (ADSA) to a biomedical development in the area of lung physiology, specifically lung surfactant, i.e., the material that coats and facilitates the functioning of the lungs of all mammals. A brief overview of lung surfactant is given first followed by a discussion of surface tension measurement techniques.

Lung surfactants are mixtures of lipids and proteins which form a thin surface film or a monolayer at the alveolar liquid-air interface. The key property of a functioning lung surfactant is its surface tension. This film modulates the surface tension of the lung, lowering the normal air-water surface tension of approximately 70 mJ/m² to extremely
low values below 1 mJ/m$^2$. By lowering and varying the surface tension during breathing, lung surfactant reduces the work needed for respiration and stabilizes the alveoli against collapse and overexpansion \[1\]. The extremely low surface tension values are difficult to measure; but certain techniques are capable of performing such measurements.

Deficiency of the lung surfactant, due to injury or lack of development in premature babies, is a relatively frequent cause of death. The lack of effective surfactant in premature babies results in neonatal respiratory distress syndrome (nRDS). This is usually treated clinically by surfactant replacement therapy using surfactant preparations such as Bovine Lipid Extract Surfactant (BLES). Acute respiratory distress syndrome (ARDS) is a frequent, life-threatening disease in which a significant increase in alveolar surface tension has been observed. However, in this case, it is known that poor performance of the surfactants used for ARDS patients is due to inhibition or inactivation of the surfactant by plasma proteins and lipids.

Drop shape techniques, based on the shape of a pendant drop, sessile drop or captive bubble, are extensively used for surface tension measurement. Early efforts in the analysis started by understanding the shapes of pendant drops and predicting the shapes for a given surface tension value \[2\]. Later, certain dimensions of the shape were tabulated along with the corresponding surface tension value. Consequently, the surface tension can be calculated through interpolation using these tables \[3\]. A more sophisticated method was later developed based on comparing a number of selected and measured points on the drop periphery to calculated drop shapes and hence determine the surface tension \[4, 5\]. An alternative method uses a semi-empirical equation to approximate surface tension from the total height and maximum diameter of a sessile drop or captive bubble \[6\].

The shape of the drop/bubble depends on the balance between gravity and surface tension. The balance between surface tension and external forces, e.g. gravity, is reflected mathematically in the Laplace equation of capillarity. When gravitational and surface tension effects are comparable, then, in principle, one can determine the surface tension
from an analysis of the shape of the drop/bubble. The surface tension tends to round the drop, whereas gravity deforms it, i.e. gravity elongates a pendant drop or flattens a sessile drop. Whenever the surface tension effect is much higher than the gravitational effect, the shape tends to become spherical in the case of both pendant and sessile drops/bubbles. Theoretically, each drop shape corresponds to a certain surface tension value.

Axisymmetric Drop Shape Analysis (ADSA) \cite{7-11} is a drop shape technique now extensively used for surface tension and contact angle measurement. ADSA, based on the shape of liquid/fluid interfaces, is complex but is adaptable to a variety of experimental circumstances including pendant drops, sessile drops and bubbles. Briefly, ADSA matches the drop/bubble profile extracted from experimental images to a theoretical Laplacian curve for known surface tension values using a nonlinear optimization procedure \cite{7-9}. An objective function is used to evaluate the discrepancy between the theoretical Laplacian curve and the actual profile. This objective function is the sum of the squares of the normal distances between the measured points (i.e. experimental curve) and the calculated curve \cite{9}. The optimization procedure minimizes the objective function and, hence, finds the surface tension value corresponding to the extracted profile from experimental images.

In this thesis, the goal is to adapt ADSA to biomedical lung surfactant development. Certain configurations of ADSA are capable of measuring the extremely low surface tension values which are observed. Although these configurations are capable and most suitable for surface tension measurement of lung surfactants, they had unexplored potential to allow for more extensive evaluation of surface properties. Three main needs arise in this area: First, access to the interface through the gas and liquid phases is needed to evaluate the role that lipids, gases and inhibitors play in the surface activity of lung surfactants. Second, a method to identify a set of characteristic properties of the surfactant preparations is needed in a way that they are independent of compression conditions and that can be used to compare the surface activity of different formula-
tions. Finally, given that the surface tension of lung surfactants changes in a dynamic experiment, it is necessary to confirm the accuracy of the surface tension measurements obtained via ADSA. Therefore, three main areas are developed in this thesis in response to these needs: First, the hardware is redesigned to allow complete access to the interface from both the air and liquid sides. Second, a detailed analysis of the dynamic surface tension measurements is used to evaluate the intrinsic properties of the lung surfactant film. These film properties are elasticity, relaxation and adsorption. Finally, the accuracy of the surface tension measurements is analyzed and has been re-evaluated. Based on this analysis, the design of certain configurations of ADSA can be optimized to facilitate more accurate surface tension measurements.

1.2 Background

In this section a detailed background is provided for the different techniques of surface tension measurements and natural lung surfactant. In Section 1.2.1, various in vitro techniques for the evaluation of surface tension of lung surfactants are discussed. Section 1.2.2 provides more background on the composition of natural lung surfactant.

1.2.1 Evaluation of Surface Tension

Various methods and techniques have been developed for evaluating the surface activity of lung surfactants. These methods usually belong to one of three categories: in vitro, in situ, and in vivo techniques. In vivo techniques involve experimentation done on the living lung of an animal. In contrast to the surface tension as an output from in vitro and in situ techniques, the main outputs of in vivo techniques are usually the lung compliance (the ability of the lungs to stretch due to a change in volume as a function of an applied change in pressure) and blood oxygenation (the partial pressure of oxygen in blood sample). Different animal models have been developed to evaluate the efficiency
of a lung surfactant preparation on premature and adult animals. In situ techniques are used to estimate the alveolar surface tension in excised lungs, i.e. in its natural setting without moving it to some special medium. In situ techniques are usually considered as an intermediate between in vivo and in vitro.

The focus of this thesis is the in vitro techniques for the evaluation of surface tension of lung surfactants. These are the most commonly used techniques for assessing their performance. In such techniques, the surface properties (such as the minimum surface tension and adsorption rates at a specific cycling conditions) of the lung surfactants are assessed by measuring the surface tension in a controlled environment experiment. In this section, various in vitro techniques are discussed.

1.2.1.1 Langmuir-Wilhelmy Balance

In a Langmuir-Wilhelmy Balance (LWB) an insoluble surfactant monolayer is spread on top of a liquid subphase filling a trough, and then compressed by means of hydrophobic barriers on the surface. During compression, surface pressure is typically monitored by a Wilhelmy plate system as shown in figure 1.1.
The net force acting on the Wilhelmy slide is the sum of forces from surface tension, gravity and buoyancy; this net force can be calculated as follows:

\[ F = 2\gamma(W + T)\cos \theta + (WTL)\rho_s g - (WT^2)\rho_w g \]  

(1.1)

where \( W, T, \) and \( L \) are the width, thickness and length of the slide, \( h \) is the wetted height of the slide, \( \gamma \) is the surface tension of the interface, \( \theta \) is the contact angle, \( g \) is the gravitational acceleration, \( \rho_s \) is the density of the slide material, and \( \rho_w \) is the density of the subphase.

More commonly, a modified expression is used giving the difference in force \( (\Delta F) \) between the case of the slide in pure subphase (no surfactant) and the case where surfactant is present at the interface,

\[ \Delta F = 2(\gamma_o - \gamma)(W + T)\cos \theta \]  

(1.2)

where \( \gamma_o \) is the surface tension of the pure subphase and \( \gamma \) is the surface tension of subphase with surfactant at the interface. This modified expression can also be expressed in terms of surface pressure,

\[ \pi = \gamma_o - \gamma = \frac{\Delta F}{2(W + T)\cos \theta} \]  

(1.3)

Equation 1.3 is usually simplified by assuming the the slide thickness \( T \) is negligible compared to the slide width \( W \), and by assuming complete wetting so that the contact angle is zero, i.e. \( \cos \theta = 1 \).

The Langmuir film balance has been widely used to study the \( \pi-A \) isotherm of many spread monolayers including octadecanol [18], dipalmitoyl-phosphatidyl-choline (DPPC) [19, 20], and dipalmitoyl-phosphatidyl-glycerol (DPPG) [21, 22]. It has been also used for adsorbed films [14, 17]. The main advantages of this method are accurate control
of molecular area, easy integration with microscopy techniques such as Brewster Angle Microscopy (BAM) and Atomic Force Microscopy (AFM), and wide commercial availability.

However, the Langmuir film balance has some limitations. First, it requires a relatively large amount of liquid sample, usually no less than several tens of milliliters. Second, it only allows relatively slow compression-expansions approximately 5 minutes per cycle [1] as fast cycling creates waves at the air-water interface, which interfere with the surface tension measurement. This limitation becomes significant in the study of lung surfactant where experiments should mimic the frequency of the normal breathing, i.e. human respiration action, at high rates of approximately 1.5 to 3 seconds per cycle [1].

The third limitation is film leakage. Since lung surfactant and phospholipid films can reach very low surface tensions (e.g. < 5 mJ/m$^2$) under dynamic compression, the liquid subphase is able to wet even very hydrophobic materials such as Teflon, the material out of which most film balances are constructed. Film leakage may occur both at the trough walls and at the barriers, either above the water level (at the air-solid interfaces) or below the water level (at the liquid-solid interfaces, in the case of soluble surfactants). Leakage at the air-solid interfaces can be reduced using tightly fitted barriers [23] or continuous Teflon ribbons [14, 24]. Leakage at the liquid-solid interfaces can be reduced by priming the Teflon walls with an alcoholic solution of lanthanum-chloride and long-chain saturated phosphatidyl-choline [16]. A fourth limitation is the difficulty of controlling the environmental conditions (temperature and humidity) during the experimentation [25].

1.2.1.2 Pulsating Bubble Surfactometer

In a Pulsating Bubble Surfactometer (PBS) [26, 32] an air bubble is formed in a surfactant suspension by drawing atmospheric air through a capillary tube as shown in figure 1.2. After adsorption of surfactant, the bubble is pulsated between two set positions. The
bubble radius is usually monitored, while the pressure in the subphase is measured by a pressure transducer. The surface tension, $\gamma$, is calculated from the measured values of the pressure drop, $\Delta P$, and the bubble radius, $R$, using the Laplace equation and assuming a spherical shape,

$$\gamma = \frac{(\Delta P)R}{2}.$$  \hspace{1cm} (1.4)

This method has several advantages: it is a simple setup and easy to use, it requires only a small volume of subphase, and it is available commercially. PBS allows fast cycling to mimic the physiological conditions. A further development of a bulk phase exchange system for PBS facilitates the study of the effect of a specific inhibitor on a preformed surfactant film [33]. This system has been used to characterize the inhibition mechanisms of different inhibitors [33, 35].

However, film leakage from the bubble surface into the capillary tube at low surface tensions is again a common complication [36]. Other limitations are the inaccurate calculation of low surface tension values due to the spherical shape assumption [27], and
the difficulty of controlling the environmental conditions (specially humidity) which are essential in lung surfactant studies.

1.2.1.3 Captive Bubble Surfactometer

In a Captive Bubble Surfactometer (CBS) [28, 37–43] an air bubble is formed in a closed chamber filled with surfactant suspension. The air bubble floats against a hydrophobic ceiling coated with 1% agarose gel as shown in figure 1.3. After film formation (spreading or adsorption), the bubble can be dynamically compressed by changing the chamber pressure.

In this case, the bubble is not expected to be spherical due to the relatively large size compared to PBS. The shape of the interface is controlled by the balance between surface tension forces and gravity as given by the Laplace equation,

\[
\gamma \left( \frac{1}{R_1} + \frac{1}{R_2} \right) = \frac{2\gamma}{R_0} + (\Delta \rho)gz
\]  

(1.5)

where \( R_1 \) and \( R_2 \) are the principal radii of curvature at a point on the interface, \( R_0 \) is
the symmetric radius of curvature at the apex point of the interface, $\Delta \rho$ is the density difference across the interface, $g$ is the local gravitational acceleration, and $z$ is the vertical distance between the apex point and the point on the interface. Therefore, the surface tension value, $\gamma$, can be determined from the shape of the interface. In CBS, the height-to-diameter ratio of the bubble is used to calculate surface tension, bubble area, and volume [40].

The CBS has all the advantages of the PBS setup and solves the problem of leakage because the bubble is not in contact with any solid surfaces. Interpretation of CBS does not assume a spherical shape. Nevertheless, the captive bubble film balance has some limitations. First, any study that requires spreading of a monolayer on the bubble interface is not trivial. Second, the small volume of the air bubble complicates the evaporation of the spreading solvent in the study of spread films; the non-evaporated portion of the solvent will influence the performance of the monolayer at the interface [44, 45]. Also, controlling the humidity in a captive bubble is relatively difficult and it is known that such environmental conditions are vital and have significant effect on lung surfactant films [46, 47]. Another limitation is that it is inoperable at the high surfactant concentrations used clinically due to visual opaqueness of lung surfactant preparations at high concentrations ($> 3$ mg/ml) [48]. Recent experiments use sub-phase spreading of highly concentrated suspensions near the interface [49]; however, the validity of that approach is yet to be determined particularly in the presence of water-soluble/dispersable surfactant inhibitors.

1.2.1.4 ADSA–CB

Axisymmetric Drop Shape Analysis (ADSA) has been used for measuring surface tension and contact angles [7–9]. Currently, there exist three constellations of ADSA for surface tension measurements: a captive bubble (ADSA–CB), a pendant drop (ADSA–PD) and a constrained sessile drop (ADSA–CSD) as shown in figure 1.4. There is also the un-
Figure 1.4: Schematic diagram of different constellations of Axisymmetric Drop Shape Analysis (ADSA).
constrained sessile drop constellation (ADSA–SD) that is used mainly for contact angle measurements \[50,52\]. As mentioned earlier, ADSA software extracts an experimental drop profile from a digitized drop/bubble image and uses an optimization procedure to match the extracted profile to a theoretical one calculated from the Laplace equation of capillarity. The surface tension of the bubble/drop is then calculated based on the matched theoretical profile. Other outputs of ADSA are drop volume, drop surface area, contact angle (if applicable), and radius of curvature at the drop/bubble apex.

The first embodiment of ADSA working in conjunction with a Captive Bubble (ADSA–CB) \[41,46,47,53,56\] has a very similar setup compared to CBS as shown in figure \[1.4\]. However, ADSA–CB uses the more accurate and automatic ADSA software for determining the surface tension, surface area and volume from a bubble image, rather than just using the height-to-diameter ratio of the bubble needed in CBS. The technique has now been widely used for soluble films \[46,47\], and also as a film balance, especially for DPPC \[44,45,57,58\].

ADSA–CB has the same advantages as CBS but provides a greater accuracy in the calculation of the surface tension and faster data acquisition and processing. On the other hand, ADSA–CB has the same limitations as CBS: the setup is not trivial especially for spread films, it is inoperable at the high surfactant concentrations (> 3 mg/ml) used clinically as the solutions are usually opaque, and controlling the humidity is relatively difficult.

### 1.2.1.5 ADSA–PD

A second embodiment of ADSA working in conjunction with a Pendant Drop (ADSA–PD) \[59,63\] has also been developed. In this method, a pendant drop of surfactant suspension is formed at the end of a teflon capillary tube as shown in figure \[1.4\]. Surface tension, surface area and drop volume are calculated by ADSA. Alternatively, a small flat circular holder (inverted pedestal) with a sharp-knife edge is used instead of the
Chapter 1. Introduction

capillary [59, 64]. This holder was introduced originally in the constrained sessile drop constellation as will be explained later, and was later used in ADSA–PD for studying surfactant films at the interface to prevent the film from spreading on to the solid surface at low surface tension. In addition to the apparent advantages of simplicity and flexibility, the pendant drop method is believed to have very high accuracy [59].

ADSA–PD has been used extensively to study the $\pi$-$A$ isotherm of several monolayers including octadecanol [59, 65], Dipalmitoyl-phosphatidyl-choline (DPPC) [62, 66, 69], and Dipalmitoyl-phosphatidyl-glycerol (DPPG) [70]. In this method, a pendant drop is formed at the end of a teflon capillary tube and a known amount of insoluble substance is spread onto its surface. The $\pi - A$ isotherms are generated by changing the drop volume in a controlled manner causing a decrease in the drop surface area and hence compression of the film. ADSA–PD is able to achieve high rates of compression and hence has advantages over a Langmuir film balance for certain studies. Nevertheless, the method still has some limitations. The deposition procedure used in the pendant drop constellation is not straightforward. This deposition can be performed in one of two ways; in both strategies, the surfactant solution is delivered through a micro syringe needle. In one strategy, the needle is brought as close as possible to the side of the hanging drop; but apparently, penetration of the needle into the bulk phase was inevitable [67] and the molecules are deposited into the bulk phase and it is uncertain whether they all diffuse to the surface. In the alternative procedure, the needle is used to deposit the surfactant solution on the teflon capillary above the pendant drop. The solution then flows down over the drop surface. Remaining surfactant molecules on the teflon capillary can lead to overestimation of the number of molecules deposited [68].

ADSA–PD was further developed to be used as a penetration film balance using an arrangement of two co-axial capillaries connected to two branches of a microinjector [60]. This system allows the bulk phase exchange after forming a stable film and was used to study the interfacial hydrolysis of mixed phospholipid monolayers by porcine pancreatic
phospholipase A$_2$ [60]. Although ADSA–PD eliminates the surfactant concentration limitation, it can suffer from film leakage at low surface tensions [59]. Another limitation at low surface tension values is that although gravity assures a well deformed drop shape, it also tends to detach the drop from the capillary holder in the case of big drops [71].

1.2.1.6 ADSA–CSD

A third embodiment of ADSA working in conjunction with a Constrained Sessile Drop (ADSA–CSD) [48, 72–75] is now extensively used for determining the dynamic surface tension of lung surfactants. In this setup, a sessile drop is formed on top of a small flat pedestal (holder) with a circular sharp knife edge preventing uncontrolled spreading and hence film leakage (figure 1.4). The sharp edge acts as a barrier to spreading. The mechanism of this barrier is readily understood in terms of what may be called Young’s inequality, a generalization of the Young equation: If the three phase line approaches an edge, Young’s equation will be obeyed. When reaching the edge, motion of the three phase line will cease and the contact line will not move until the drop has become much larger. During this time, the apparent contact angle increases. When the contact angle becomes so large that it becomes equal to the Young angle on the adjacent surface, motion of the three phase line will continue, and, from the point of view of the surface tension measurement, undesirable processes occur, such as film spreading. However, there will be a large range of drop sizes over which the volume of the drop can be changed at virtually constant drop diameter, offering an opportunity for film balance type of work. Hence, the apparent contact angle will change with the drop volume.

This holder was introduced originally for the study of density and surface tension of polymer melts [73]. Due to the presence of the sharp edge, ADSA–CSD proved to be very suitable for a wide range of surface tension measurements including very low values, down to below 1 mJ/m$^2$, that are difficult to measure with other setups. ADSA–CSD is used to perform dynamic cycling by successive compression/expansion of the drop via
programmed cycling of the drop volume. This is usually done through several cycles (normally 20) with prescribed periodicity and compression ratio (percentage of surface area reduction). In such experiments, a large amount of data is collected including the surface tension as a function of time as well as the change of the drop surface area and volume.

ADSA–CSD has been used so far only for the study of films adsorbed from solution (soluble films). Matters such as the effect of humidity on film formation [25], and the development of polymeric additives for clinical lung surfactants [76] have been considered. ADSA–CSD studies were performed at the high rates of compression which mimic human breathing and which are not achievable using a Langmuir setup. A specially-designed environmental control chamber for ADSA–CSD facilitates the control of gas composition, temperature and humidity to mimic the physiologically relevant conditions.

ADSA–CSD is believed to be free of all restrictions and limitations of other conventional methods. The main advantages are that it is very simple to setup and is easy to use and only small liquid volumes (microliter drops) are required to perform very accurate surface tension measurements. In the context of lung surfactants, ADSA–CSD is capable of simulating physiological conditions in the lungs, i.e. the cycling rate to simulate breathing, environmental and temperature conditions. However, ADSA–CSD has no concentration limitation of surfactant suspension. Other unexplored potentials: it is possible to perform high frequency cycling and it is possible to access the air/liquid interface from both sides to allow spreading and bulk replacement. Although such potential would be useful, especially in the lung surfactant context, it has not been explored so far.

1.2.2 Pulmonary Surfactants

Pulmonary surfactants are mixtures of lipids and proteins forming a monolayer at the alveolar liquid-air interface. Pulmonary surfactant consists of about 90% lipids and about
10% proteins. Four surfactant apoproteins are present in native surfactants, called SP-A, SP-B, SP-C, and SP-D. Approximately 10-20% of the lipids are neutral, and the rest (80-90%) are phospholipids (PL). Phosphatidylcholine (PC) is the predominant PL species (about 80%) followed by phosphatidylglycerol (PG) which comprises more than 10% of PL. Dipalmitoylphosphatidylcholine (DPPC) comprises about 50% of PC, i.e., about 40% of the total PL. The other main lipid component is dipalmitoylphosphatidylglycerol (DPPG) which comprises almost 10-40% of PG [1, 36, 77, 78]. Native human lung surfactant is near the high end of these percentages [78].

A brief review of the commercial exogenous lung extracts is also provided in Appendix A. Commercial lung extracts are divided into two main categories according to the source of the preparation: natural lung surfactants which contain exogenous lung extracts from animals and synthetic lung surfactants which do not contain any natural extracts. In this thesis, a clinical exogenous lung surfactant called Bovine Lipid Extract Surfactant (BLES) is chosen as a model lung surfactant. BLES is made from lung surfactant lavaged from cows and is produced locally in Canada. A detailed list of other clinically used lung surfactants worldwide is given Appendix A. It is noted that DP-PC/PG mixtures are used as the main component of many clinical synthetic exogenous lung surfactants, e.g., ALEC (artificial lung expanding compound) [79, 81], Surfaxin (KL₄ or lucinactant) [82, 84], and Venticute (Recombinant SP-C or lusupultide) [85, 87].

1.3 Scope and Purpose of Thesis

The objective of this thesis is to further develop and automate ADSA–CSD to allow further studies of lung surfactants. As discussed in Section 1.2.1, ADSA–CSD can be applied – in principle – to lung surfactant studies at relevant physiological conditions which include appropriate temperature, relative humidity, gas composition, pollution, surfactant concentration, respiration periodicity, and compression ratio. Until now, ADSA–CSD
has been used only for the study of films adsorbed from solution (soluble films) and matters such as the effect of humidity on film formation [25], and the development of polymeric additives for clinical lung surfactants [76] have also been considered. However, ADSA–CSD had some unexplored and undiscovered potential which will be addressed here. The use of ADSA as a film balance was so far done using the pendant drop constellation and there were considerable experimental difficulties [59] that can be avoided in a constrained sessile drop setup. In this thesis, ADSA–CSD is redesigned to allow access to the air-liquid interface from either side.

In clinical reality, lung surfactant films can be altered from both the airspace and the bulk liquid phase that carries the film. Contaminations (pollution) affect the surfactant interface from the air side and treatment (surfactant therapy for premature babies) is instilled from the air side, while leakage of plasma and blood proteins usually occurs from the liquid side, causing severe lung injury. Such inhibitors degrade the surface activity of the surfactant by impairing the adsorption and the surface tension lowering abilities of the surfactant. Therefore, accessing the lung surfactant interface from both sides is a necessary feature in a general in vitro technique used for the assessment of the surface activity of lung surfactant. In Chapter 2, ADSA–CSD is redesigned to be used as a micro film balance allowing access to the interface from both the gas-side and the liquid-side. This allows deposition from the gas side as well as complete exchange of the bulk liquid phase. The key feature of the design is the insertion of a second capillary into the bulk of the drop to facilitate addition or removal of a secondary liquid. The new design is used to study lung surfactant inhibition resistance (subphase injection of inhibitor under an uninhibited film) and inhibition reversal (subphase injection of surfactant under an inhibited film). The access from the air-side is illustrated by a series of experiments of the collapse surface pressure of spread pure monolayers of DPPC and DPPG (the main constituents of lung surfactants).

To assess the performance of surfactant preparations subject to dynamic compres-
sion/expansion, typically only the minimum surface tension at the end of compression is reported. While the minimum surface tension is useful for a preliminary assessment, it must be realized that it depends on the compression protocol [46]. Therefore, it is necessary to concentrate on the properties of the film itself such as its elasticity, its formation (adsorption) and its stability (or relaxation tendency) in addition to, if not in place of, the minimum surface tension. In Chapter 3 a dynamic compression-relaxation model (CRM) is developed to describe the mechanical properties of lung surfactant films by investigating the response of surface tension to changes in surface area. The model treats the compression/expansion with the conventional definition of dilatational elasticity of insoluble surfactants, and treats the relaxation and adsorption of the film as first order processes driven by the excess surface free energy of the film. The model is used to evaluate the quality of lung surfactant preparations based on calculating the film properties independent of the compression protocol used.

The accuracy of the determined mechanical properties of lung surfactant films depends on the accuracy of the measured response of surface tension to changes in surface area. Therefore, high accuracy surface tension measurements are necessary in such studies. It is well documented that the accuracy of the surface tension determined by ADSA can depend on drop size, unless all drops of different sizes are well deformed and far from spherical [64]. A detailed analysis of constrained sessile drop shapes and the accuracy of the measured surface tension values is developed using a shape parameter concept in Chapter 4. Dimensional analysis is used to describe similarity in constrained sessile drop shapes and to express the problem using appropriate dimensionless groups. Based on this analysis, the design of ADSA–CSD is optimized to facilitate more accurate surface tension measurements. The validity analysis is further extended to the pendant drop (ADSA–PD). This setup is the most frequently used and most accurate drop shape technique for surface tension measurement outside the area of lung surfactants.
Chapter 2

ADSA-CSD as Micro Film Balance*

2.1 Introduction

ADSA–CSD has been extensively used for the study of lung surfactants [91]. Matters such as the effect of humidity on film formation [25], and the choice of polymeric additives for clinical lung surfactants [76] have been considered. ADSA–CSD studies were performed at high rates of compression (to mimic human breathing in lung surfactant studies). A specially-designed environmental control chamber for ADSA–CSD facilitates the control of gas composition, temperature and humidity to mimic the physiologically relevant conditions [25]. ADSA–CSD is believed to be free of all restrictions and limitations of other methods as mentioned in detail in Chapter 1.

However, certain studies cannot be readily performed using the current ADSA–CSD setup. In the lung surfactant context, certain insoluble molecules such as DPPC and DPPG are closely related to the surface activity of pulmonary surfactant as shown in Chapter 1. The study of these insoluble molecules is essential in understanding the surface properties of lung surfactants. Studying lung surfactant inhibition is also an important aspect. Such studies are introduced here by removing the bulk phase, after

*Portions of this chapter were previously published in Refs. [88–90], reproduced with permission.
forming a sessile drop of a basic lung surfactant preparation, and exchanging it for one containing different inhibitors. This mimics the leakage of plasma and blood proteins into the alveolar spaces altering the surface activity of lung surfactant in a phenomenon called surfactant inhibition. These studies cannot be performed using the current ADSA–CSD setup.

In response to these needs, two main capabilities are added to the ADSA–CSD setup. The first one is the deposition capability allowing deposition of molecules onto the air/water interface from the air side. This allows the study of the collapse of deposited films through the study of pure monolayers as well as mixed monolayers. DPPC and DPPG monolayers are studied here to characterize the role of such molecules in maintaining stable film properties and surface activity of lung surfactant preparations. The second capability is the double injection, facilitating the complete exchange of the subphase of a spread or adsorbed film from the liquid side. This feature allows certain studies relevant to lung surfactant research that cannot be readily performed by other means. The development will be illustrated through studies concerning lung surfactant inhibition.

The redesigned ADSA–CSD is a versatile tool for surface tension measurements that has a wide range of applications outside the scope of the lung surfactant research area. For example, the deposition capability allows for the study of properties of insoluble films such as film relaxation and collapse and interfacial dilational elasticity. The double injection capability allows studies on the interaction between a monolayer and a surfactant dissolved in the subphase.

### 2.2 Deposition Capability

The collapse pressure is the highest surface pressure to which a monolayer can be compressed without detectable expulsion of molecules into a new phase [92]. Experimentally, it was found that many monolayers can be compressed to pressures considerably
higher than their equilibrium spreading pressure [11, 92, 93]. Eventually, however, it was found impossible to increase the surface pressure further. If compression continues, the monolayer collapses ejecting surfactant molecules into one or more surface or subsurface collapse structures or phases [11, 93]. Monolayer collapse is accompanied by a change in slope or by a horizontal plateau in the surface pressure/area $\pi$-$A$ isotherm. The surface pressure, $\pi$, can be computed by calculating the difference between the surface tension of the pure liquid (without an insoluble monolayer spread at the interface), $\gamma_0$, and the surface tension with the monolayer spread at the interface, $\gamma$, i.e. $\pi = \gamma_0 - \gamma$.

These isotherms are measured with a surface balance or a film balance. Film balances have been used to study monolayer properties for different substances, such as the collapse pressure. The collapse pressure is used as an indication of the monolayer stability; the higher the collapse pressure, the more stable the monolayer. The first film balance was developed by Langmuir [12] and modified subsequently by others [92, 93]. The Langmuir film balance suffers from three main limitations as indicated in detail in Chapter 1. First, it requires a relatively large amount of liquid sample. Second, it only allows relatively slow compression-expansions. Third, it suffers from film leakage especially at very low surface tensions (very high surface pressures). These limitations become significant in the study of lung surfactant.

The pendant drop and the captive bubble techniques have also been used to study the $\pi$-$A$ isotherm of several monolayers as shown in Chapter 1. Nevertheless, both techniques have one or more of the following limitations when used as a film balance. In both techniques, the deposition procedure to spread a monolayer on the bubble/drop interface is neither straightforward nor trivial. In the pendant drop technique, film leakage usually occurs at low surface tensions (high surface pressures); and gravity tends to detach the drop from the capillary holder in the case of big drops [71]. In the captive bubble technique, controlling the humidity is a difficult matter. More details regarding these limitations were given in Chapter 1.
ADSA–CSD has been used so far only for the study of films adsorbed from solution (soluble films). Here, the usage of ADSA–CSD as a film balance is illustrated. The fact that the drop is sitting on a pedestal makes the deposition near the drop apex much easier compared to the case of the pendant drop. Another feature is that gravity will cause the drop to be well deformed at very low surface tension values; this guarantees high accuracy of ADSA calculations, especially in the vicinity of the collapse pressure, where very low surface tension values exist. Using ADSA–CSD, the collapse region will be studied carefully to gain insight into the collapse details of three different monolayers: octadecanol, dipalmitoyl-phosphatidyl-choline (DPPC), and dipalmitoyl-phosphatidyl-glycerol (DPPG).

The reason for choosing DPPC and DPPG is their relevance to pulmonary surfactant studies as shown in Chapter 1. In the literature, a DPPC/DPPG mixture ratio of 80:20 was used in several studies to mimic natural lung surfactant lipids [94–98]. This ratio is usually selected because it is similar to the ratio found in a variety of mammalian lung surfactant extracts. Other studies have used a ratio of 70:30 [99, 100]. Most of these studies (using a ratio of 80:20 or 70:30) have focused on investigating the effect of surfactant proteins (SP-A, SP-B, and SP-C) on the properties of a model lung surfactant composed of only DPPC and DPPG at the specified ratio. Properties at the very high surface pressures which occur in the lungs were not investigated in any of these studies. DPPC/PG mixtures are also used as the main component of many clinical synthetic exogenous lung surfactants, e.g. ALEC (artificial lung expanding compound) [79, 81], Surfaxin (KL4 or lucinactant) [82–84], and Venticute (Recombinant SP-C or lusupultide) [85, 87]. In ALEC, DPPC (70%) is the main component for lowering surface tension, while egg PG (30%) is used to improve adsorption and spreading [79].

There appears to be no systematic study to date to study the effect of increasing the DPPG content in a DPPC/DPPG mixture on the ultimate collapse pressure. Here, ADSA–CSD is also used to study spread monolayers of mixed DPPC/DPPG films. Com-
pression isotherms were obtained for different DPPC/DPPG mixture ratios with special focus on the ultimate collapse pressure, film elasticity and film hysteresis for every mixture ratio.

2.2.1 Experimental Details

2.2.1.1 Materials and Methodology

1-Octadecanol was purchased from Sigma-Aldrich (Fluka) Co. (cat. 74720) with a purity $\geq 99.0\%$; dipalmitoyl-phosphatidyl-choline (DPPC) from Sigma-Aldrich Co. (cat. P-4329) with a purity $\geq 99.0\%$; and dipalmitoyl-phosphatidyl-glycerol [ammonium salt] (DPPG) from Sigma-Aldrich Co. (cat. P-5650) with a purity $\sim 99.0\%$. All amphiphilic substances were used without further purification.

Heptane, ethanol, chloroform and methanol were used as spreading agents. Heptane was purchased from EMD Co. (cat. HX0082-1) with a purity 99.6\% (99.95\% saturated C7 hydrocarbons); ethanol from Commercial Alcohols Inc. (cat. UN-1170); chloroform from Sigma-Aldrich Co. (cat. 36,692-7) with a purity $\geq 99.8\%$; and methanol from Sigma-Aldrich Co. (cat. 27,047-4) with a purity 99.93\%.

Ocatadecanol was dissolved in a heptane/ethanol (9:1 v/v) mixture to form a stock solution with a concentration of 1.2270 mg/ml and then diluted in heptane only to a concentration of 0.0258 mg/ml. DPPC was dissolved in a heptane/ethanol (9:1 v/v) mixture to form a stock of concentrated solution and then diluted in a heptane/ethanol (9:1 v/v) mixture to different concentrations in the range of 0.0025 to 0.0250 mg/ml. DPPG was dissolved in a chloroform/methanol (9:1 v/v) mixture to form a stock of concentrated solution and then diluted in a heptane/ethanol (9:1 v/v) mixture to different concentrations in the range of 0.0025 to 0.0250 mg/ml. The stock and dilute solutions were always freshly prepared on the day of experiment and subsequently mixed (in the case of the mixed monolayers study) to give the required mixture DPPC/DPPG ratio.
and used in the experiments. The water used as the subphase in the experiments is
demineralized and glass distilled (pH $\simeq 5$); the ionic strength is almost negligible.

A schematic diagram of the experimental setup for ADSA–CSD is shown in figure 2.1. As shown, a constrained sessile drop is formed on a stainless steel pedestal with an outside
diameter of 6.2 mm and an inside diameter of 1.5 mm. A Cohu 4800 monochrome camera
is mounted on a Wild-Apozoom 1:6 microscope. The video signal of the constrained
sessile drop is transmitted to a digital video processor, which performs the frame grabbing
and the digitization of the image.

A computer is used to acquire images from the image processor and to perform image
analysis and computation. The rate of image acquisition for the present experiments is
ten images per second. ADSA software extracts an experimental drop profile from the
digitized image and uses an optimization procedure to match the extracted profile to a
theoretical one calculated from the Laplace equation of capillarity. The surface tension of
the constrained sessile drop is then calculated based on the matched theoretical profile.
Other outputs of ADSA are radius of curvature, drop volume and drop surface area. The
fact that we can measure changes in surface area makes this methodology suited for film
balance experiments.

The volume of the drop is controlled by a syringe connected to a stepper motor.
By moving the syringe plunger, changing the volume changes the surface area of the
constrained sessile drop. Although the surface area is changed indirectly, this change is
Figure 2.2: The change of surface tension with time after the deposition of octadecanol in heptane/ethanol solution.

essentially linear for the relatively small changes in volume [59].

2.2.1.2 Experimental Procedure

A drop of pure water was formed on the stainless steel pedestal which is connected to the syringe and the stepper motor. A few images were acquired and analyzed by ADSA, providing the surface tension of the pure water subphase, $\gamma_o$. All experiments were performed at room temperature of 24°C.

Using a Hamilton microliter syringe with 0.01% accuracy and a micro-manipulator, a known amount (typically 5 $\mu$l) of the diluted surfactant solution was deposited on top of the sessile drop. After the deposition, complete evaporation of the spreading agent takes place in approximately 60 seconds. This is shown in figure 2.2 where the surface tension
Table 2.1: Experimental parameters used for different monolayer types

<table>
<thead>
<tr>
<th>Monolayer</th>
<th>Volume (µl)</th>
<th>Concentration (mg/ml)</th>
<th>(10^{-14} \times \text{No. of Molecules})</th>
<th>Area Rate (cm²/min)</th>
<th>Area Rate (Å²/molecule-min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octadecanol</td>
<td>5.0</td>
<td>0.0258</td>
<td>2.87</td>
<td>0.19</td>
<td>6.65</td>
</tr>
<tr>
<td>DPPC</td>
<td>5.0</td>
<td>0.0250</td>
<td>1.03</td>
<td>0.19</td>
<td>18.94</td>
</tr>
<tr>
<td>DPPG</td>
<td>5.0</td>
<td>0.0250</td>
<td>1.02</td>
<td>0.19</td>
<td>19.47</td>
</tr>
</tbody>
</table>

of pure water decreases after deposition of octadecanol in heptane/ethanol solution and then drifts back up again as the spreading agent evaporates. In the actual experiments, 3 minutes are allowed to elapse to ensure complete evaporation of the spreading agent before the actual compression is started.

A crucial part of the experiment is the deposition of the insoluble surfactant onto the drop surface. The constrained sessile drop constellation used here makes the deposition procedure much easier compared to deposition methods in the pendant drop constellation. A detailed description of the deposition method used in the current experiments is shown in figure 2.3. Increasing the size of the drop after the deposition promotes complete spreading of a monolayer over the whole surface of the drop. After measuring the surface tension of a pure water drop at full size, the size of the drop is decreased from the full size (∼80µl) to half size (∼40µl) before deposition. Then the solution deposition (5µl) is performed from the syringe needle over the water drop apex. Two or more solution drops fall from the needle onto the drop, causing more than one minimum in the surface tension in figure 2.2. In some cases, part of the solution dose remains attached to the needle as a pendant drop; in such cases the needle is moved closer to the drop without penetrating the interface. The sessile drop is expanded back to full size right after deposition.

At this point, the constrained sessile drop carries an insoluble monolayer and is then covered with a glass cuvette to isolate the drop from the environment and to prevent contamination. The surface area was then decreased by reducing the drop volume with the motor-controlled syringe. For the first study of pure monolayers, table 2.1 summa-
Figure 2.3: The process of solution deposition on a constrained sessile drop: (a) the drop at the full size (∼80µl) before deposition; (b) the drop at half size (∼40µl) before deposition; (c) solution deposition (5µl) where more than one solution drop falls from the needle over the apex; (d) part of the solution is still attached to the needle; (e) the needle is moved closer without penetrating the interface; (f) the sessile drop after complete deposition; (g) expansion of the sessile drop back to the full size right after deposition; (h) the sessile drop after complete solvent evaporation (∼2min).
Chapter 2. ADSA-CSD as a Micro Film Balance

izes the amounts of deposition as well as the compression rates for different surfactant
types calculated based on equations 2.1 and 2.2 below. For the second study of mixed
monolayers, the deposition amount for all mixtures used is 5.5 µl volume with a mix-
ture concentration of 0.025 mg/ml. The compression rate is fixed at 0.19 cm²/min for
all experiments. During the compression, images were acquired and saved for further
analysis.

All acquired images were analyzed by ADSA, providing the surface tension value,
\( \gamma \), the drop surface area, \( A \), the drop volume, \( V \), and the radius of curvature at the
apex, \( R_c \). Figure 2.4 shows a typical result of ADSA for the deposition of octadecanol
in heptane/ethanol solution on a water drop. The number of molecules deposited can be
calculated

\[
\text{Molecules} = \frac{CV_dA_v}{M_w}
\]  

(2.1)

where \( C \) is the concentration of the dilute solution used, \( V_d \) is the volume used in the
deposition, \( A_v \) is Avogadro’s number, and \( M_w \) is the molecular weight of the specific
surfactant. The change of the surface area with time is used to calculate the rate of
molecular area compression

\[
\frac{\text{Area}}{\text{Molecules} \times \text{Minutes}} = \left( \frac{1}{\text{Molecules}} \right) \left( \frac{\text{Area}}{\text{Minutes}} \right)
\]  

(2.2)

The parameters of all experiments are summarized in Table 2.1. The value of the surface
pressure, \( \pi \), can be readily computed by calculating the difference between \( \gamma_o \) and \( \gamma \), i.e.

\[
\pi = \gamma_o - \gamma
\]  

(2.3)

For the mixed monolayer study, there was an interest to see the effect of mixing
DPPC and DPPG on the film elasticity. The dilatational elasticity, \( \epsilon \), is defined as the
magnitude of surface tension reduction (or surface pressure increase) for a given reduction
Figure 2.4: The change of surface tension, drop surface area, drop volume and radius of curvature at the apex with time of a constrained sessile drop of an octadecanol monolayer.
of the relative surface area of the film. The film elasticity can be calculated from the change in surface pressure resulting from a small change in drop surface area:

$$\epsilon = \left| \frac{d\pi}{d \ln A} \right| = \left| A \frac{d(\gamma_o - \gamma)}{dA} \right|$$

(2.4)

where $\pi$ is the surface pressure, $\gamma$ and $\gamma_o$ are the surface tension values in the presence and absence of a phospholipid film, respectively, and $A$ is the drop surface area.

### 2.2.2 Results for Pure Monolayers

Figure 2.5 shows the change of surface pressure with area per molecule for an octadecanol monolayer at a compression speed of 0.19 cm$^2$/min (6.65 Å$^2$/molecule·min). Figure 2.5(a) shows two runs, illustrating reproducibility. Although ten images were acquired every second, here only two points per second are shown to enhance graph readability. This applies for figures 2.5, 2.6 and 2.8. However, figure 2.7 shows the raw data acquired from ADSA–CSD at the rate of ten images per second. As shown in figure 2.5(a), the surface pressure starts near 0 mJ/m$^2$ and reaches approximately 61 mJ/m$^2$ as the film is compressed. Further compression did not raise the surface pressure. A plateau is observed indicating collapse of the monolayer at this limiting surface pressure. Further inspection shows that there is a change in the isotherm slope (in the domain of rapid increasing area, i.e. the liquid condensed phase) starting well below the limiting collapse pressure. Figure 2.5(b) shows the isotherm of five different runs in the vicinity of the collapse pressure, where point $A$ indicates the point where the slope of the isotherms starts to change.

Figure 2.6 shows the change of surface pressure with area per molecule for a DPPC monolayer with a compression speed of 0.19 cm$^2$/min (18.94 Å$^2$/molecule·min). Figure 2.6(a) shows a typical DPPC isotherm; the surface pressure starts at approximately 5 mJ/m$^2$ and reaches about 70 mJ/m$^2$ as the film is compressed. A plateau is observed
Figure 2.5: The change of surface pressure with area per molecule of a constrained sessile drop of an octadecanol monolayer: [a] the full isotherm for different runs; [b] the isotherm of five different runs in the vicinity of the collapse pressure (only two points per second shown).
Figure 2.6: The change of surface pressure with area per molecule of a constrained sessile drop of a DPPC monolayer: (a) the full isotherm; (b) the isotherm in the vicinity of the collapse pressure (only two points per second shown).
indicating the collapse of the monolayer at this very high limiting surface pressure (very low surface tension, approximately 2 mJ/m$^2$). Such low surface tension was not achievable using a pendant drop \cite{62, 66-69}, where gravity will detach the drop at very low surface tension values. Further inspection shows that there is a change in the isotherm slope starting at pressures well below the limiting collapse pressure. This is illustrated in figure \ref{fig:2.6(b)} which shows the isotherm in the vicinity of the collapse pressure, where point $A$ ($\simeq 52.8$ mJ/m$^2$) indicates the point where the isotherm slope changes.

As shown in figure \ref{fig:2.6(b)}, the isotherm shows a continuous change starting from point $A$ till the horizontal plateau indicated by the dotted line. To further understand the isotherm behaviour in this region, we show in figure \ref{fig:2.7} the change of surface tension and drop surface area with time in two key regions; the first is the vicinity of point $A$ shown in figure \ref{fig:2.7(a)} and the other is the vicinity of the isotherm horizontal plateau shown in figure \ref{fig:2.7(b)}.

In the first region, figure \ref{fig:2.7(a)}, the surface tension shows episodes of collapse in terms of sudden changes. The curve before the time window shown is quite smooth. These changes are in the range of 2 mJ/m$^2$, while it was shown \cite{101} that the error of the measurement is less than 0.1 mJ/m$^2$. These episodes of collapse are accompanied by a slight decrease in the drop surface area. The reason is that for the same drop volume, when the surface tension increases at a collapse episode, the drop becomes more spherical, decreasing the drop surface area at a given volume.

In the second region, figure \ref{fig:2.7(b)}, the surface tension is almost constant at a very low value corresponding to the horizontal plateau in the surface pressure isotherm. The most interesting part in this figure is the change of drop surface area with time at this very low surface tension. In this region, further compression cannot increase the surface pressure anymore indicating an ultimate collapse pressure. However, due to the continuous compression, the drop surface area shows sudden drops. This suggests that molecules are leaving the interface to the bulk causing a sudden drop in the surface area. This aspect
Figure 2.7: The change of surface tension and drop surface area with time of a constrained sessile drop of a DPPC monolayer: (a) in the vicinity of initial episodes of collapse; (b) in the vicinity of the ultimate collapse pressure (raw data acquired at the rate of ten images per second shown).
Table 2.2: Collapse pressure (mJ/m\(^2\)) from different runs of different monolayer types and the average value along with the standard deviation

<table>
<thead>
<tr>
<th>Monolayer</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Run 4</th>
<th>Run 5</th>
<th>Average</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial Episode of Collapse</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Octadecanol</td>
<td>54.8</td>
<td>53.5</td>
<td>54.5</td>
<td>55.7</td>
<td>54.1</td>
<td>54.5</td>
<td>0.37</td>
</tr>
<tr>
<td>DPPC</td>
<td>52.8</td>
<td>48.5</td>
<td>44.8</td>
<td>44.2</td>
<td>-</td>
<td>47.9</td>
<td>1.98</td>
</tr>
<tr>
<td>DPPG</td>
<td>57.8</td>
<td>58.5</td>
<td>59.7</td>
<td>57.4</td>
<td>-</td>
<td>58.4</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>Ultimate Collapse Pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Octadecanol</td>
<td>61.5</td>
<td>62.1</td>
<td>61.9</td>
<td>60.7</td>
<td>60.3</td>
<td>61.3</td>
<td>0.34</td>
</tr>
<tr>
<td>DPPC</td>
<td>70.5</td>
<td>70.2</td>
<td>70.1</td>
<td>70.0</td>
<td>-</td>
<td>70.2</td>
<td>0.12</td>
</tr>
<tr>
<td>DPPG</td>
<td>59.9</td>
<td>58.2</td>
<td>60.7</td>
<td>57.2</td>
<td>-</td>
<td>59.0</td>
<td>0.80</td>
</tr>
</tbody>
</table>

will be treated in more detail in the discussion section.

Figure 2.8 shows the change of surface pressure with area per molecule for a constrained sessile drop of a DPPG monolayer with a compression speed of 0.19 cm\(^2\)/min (19.47 Å\(^2\)/molecule·min). Figure 2.8(a) shows a typical DPPG isotherm; the surface pressure starts at approximately 3 mJ/m\(^2\) and reaches about 59 mJ/m\(^2\) as the film is compressed. A plateau is observed indicating the collapse of the monolayer at this limiting surface pressure. In contrast to octadecanol and DPPC, DPPG did not show a sudden change in the isotherm slope before the ultimate collapse pressure. Figure 2.8(b) shows the isotherm in the vicinity of the collapse pressure, where point A (≃ 58.5 mJ/m\(^2\)) indicates the first point of the change of the isotherm slope.

To illustrate the reproducibility of the results, every experiment was repeated 4 or 5 times. Table 2.2 summarizes the values of the first episode of collapse as well as the ultimate collapse pressure from different runs for the different monolayers (Octadecanol, DPPC and DPPG) and the average values along with the standard deviation.
Figure 2.8: The change of surface pressure with area per molecule of a constrained sessile drop of a DPPG monolayer: [a] the full isotherm; [b] the isotherm in the vicinity of the collapse pressure (only two points per second shown).
2.2.3 Discussion for Pure Monolayers

2.2.3.1 Problem of leakage

The results of compressing an octadecanol monolayer are in good agreement with previous experiments with the pendant drop constellation [59, 65]. The new methodology proves to be comparable with existing methods with respect to accuracy and reproducibility. ADSA–CSD shares certain advantages with ADSA–PD over Langmuir film balances. One of the key advantages is the ability to use higher compression rates, and just as the Langmuir balance, there is no lower limit for the rate of compression and expansion. Another potential strength is that environmental control (contamination, humidity, pressure and temperature) is straightforward and easy to integrate with the current setup; this will be a necessity, e.g. for lung surfactant studies [25, 76]. Other advantages are that only small quantities of liquid and insoluble surfactants are required, and that a very simple setup is needed and could be integrated with microscopy systems, e.g. BAM or AFM, for further studies. Compared to ADSA–PD, ADSA–CSD has an easier deposition procedure.

However, the main advantage here of ADSA–CSD over ADSA–PD and Langmuir balance is that leakage is eliminated completely. Leakage in the pendant drop can affect the manifestation of the collapse pressure as a horizontal plateau in the surface pressure/area isotherm. For example, previous results using a pendant drop [65] have shown the presence of the horizontal plateau for some octadecanol isotherms, while a sudden decrease in the surface pressure after a certain point was shown for higher compression speeds. This point was originally considered as the collapse pressure. However, the effect was recently attributed to monolayer leakage on the teflon capillary supporting the pendant drop [88]. In fact, all of the studies performed using a pendant drop with a teflon capillary [59, 62, 65, 70] could not achieve any surface pressures larger than about 62 mJ/m². Although leakage in pendant drops can be eliminated by using a sharp edge
holder as will be discussed in Chapter 4, the problem with low surface tension values is partially due to the fact that the gravity forces are more pronounced at these low surface tension values (high surface pressure values) and would act to detach the drop from the holder. The current constrained sessile drop setup is free of any of these shortcomings. It has the ability to measure very low surface tension values as shown in the DPPC results, and it features much easier deposition procedure and leak-proof design. A further merit of ADSA–CSD is the ability of performing high rates of compression, e.g. for lung surfactant studies [25, 76], see Chapter 3.

### 2.2.3.2 Onset of collapse versus ultimate collapse pressure

Disagreements between investigators about collapse pressure has been considerable [65]. Some consider the surface pressure at which the isotherm slope starts to change to be the starting point of ejection of molecules from the interface as the collapse pressure in agreement with the definition given in the introduction [92]. Others would consider a horizontal plateau in the isotherm as the collapse pressure, while an intermediate point in between those two case is sometimes also considered [1].

The results presented here show a distinctive difference between the onset of collapse and the ultimate collapse pressure (ultimate strength) of these films. For example, compression of a DPPC monolayer causes the isotherm to change its slope at a certain point as shown in figure 2.6(b). This change in the isotherm slope reflects the existence of some interaction between molecules at the interface causing some episodes of collapse. This point is called here the onset of collapse. Upon further compression, the monolayer adapts and sustains higher surface pressure until reaching a maximum or ultimate strength or an ultimate collapse pressure. Thus, a collapse region can be defined to start with an initial episode of collapse (onset of collapse) and to end when the ultimate (or maximum) collapse pressure is reached, manifested in a horizontal plateau in the surface pressure/area isotherm. From an applied perspective, e.g. for lung surfactant, we are
mostly interested in knowing if the film can sustain high surface pressures (e.g. \( > 67 \text{ mJ/m}^2 \)), i.e. surface tensions that prevent lung collapse.

Whereas a Langmuir film balance controls the surface area and measures the change in surface pressure, ADSA–CSD measures the change of both surface area and surface tension with time caused by the externally imposed change of drop volume. This feature allows ADSA–CSD to provide a detailed study of the collapse region, as shown in figure 2.7 for DPPC. Figure 2.7(a) shows the change of surface tension and drop surface area with time in the vicinity of the onset of collapse, while figure 2.7(b) focusses more on the vicinity of the ultimate collapse pressure. Episodes of collapse occur as indicated by the change of surface tension in the former; however, the surface tension is almost constant in the latter. The change of drop surface area provides more information in both figures.

In figure 2.7(a) the episodes of collapse are accompanied by some irregularities in the surface area. In figure 2.7(b) while the surface tension is constant, the surface area shows sudden decreases at some points. The sudden decrease in area suggests squeeze out of some molecules from the interface. The decrease in surface area can be used to estimate the size of regions and the number of molecules that are being pushed into the subphase. For example, from figure 2.7(b) at time 148 seconds the area suddenly drops from 0.300 cm\(^2\) to 0.295 cm\(^2\). Since the surface tension is essentially constant, this suggests that a region (or regions) of accumulative area equal to approximately 0.005 cm\(^2\) or \(5 \times 10^{13} \text{ Å}^2\) have been pushed out to the subphase. The minimum area per DPPC molecule is known to be in the range of 35 to 40 Å\(^2\)/molecule; this gives an estimate of the number of molecules that have to leave the interface as in the order of \(10^{12}\) molecules, i.e. 1% of the total material deposited. Such information might be of specific interest, e.g. for lung surfactant systems. Obviously, such information cannot be obtained by a Langmuir film balance.
2.2.3.3 Comparison of the three systems

Table 2.2 summarizes the values obtained for onset of collapse and ultimate collapse pressure for the three systems studied here: octadecanol, DPPC and DPPG. Octadecanol and DPPC show a distinctive difference between the onset of collapse and the ultimate collapse pressure, while both values are very close for DPPG. In fact, some of the DPPG runs (run 2 and 4) did not show any sudden change in the isotherm slope until the ultimate collapse pressure was reached, i.e. there was no separate distinctive onset of collapse for these runs, as shown in figure 2.8. The values for the ultimate collapse pressure were well reproducible for all three systems. However, the onset of collapse is harder to reproduce especially for the DPPC monolayer as reflected in the high value of the standard deviation.

Octadecanol was found to have an ultimate collapse pressure of 61.3 mJ/m$^2$, DPPC of 70.2 mJ/m$^2$, and DPPG of 59.0 mJ/m$^2$. The superior ability of DPPC to sustain such a high pressure may help to understand why it is the main component of lung surfactant. The difference between the ultimate collapse pressure of these three systems is believed to be due to several factors, including the molecular structure, the chain length, and the properties of the head group. DPPC $\text{[C}_{40}\text{H}_{80}\text{NO}_8\text{P]}$ has two aliphatic chains with a large choline head group, compared to octadecanol molecules $\text{[CH}_3\text{(CH}_2)_{17}\text{OH]}$ with a single alkyl chain and only a small head group. DPPG $\text{[C}_{38}\text{H}_{75}\text{O}_{10}\text{P-NH}_3]$ has a similar molecular structure to DPPC; however, unlike DPPC which is zwitterionic, DPPG is anionic. Further discussion of the collapse of each system is given below.

Results of Brewster Angle Microscopy (BAM) of octadecanol monolayers have shown that under the usual spreading conditions, irregularly shaped islands of condensed phase are distributed in a continuous gaseous phase $\text{[65]}$. Upon compression, these islands eventually touch each other and start to coalesce and rearrange. With increasing surface pressure, the condensed phase becomes a continous phase with irregular areas of the residual gaseous phase $\text{[65]}$. However, results presented here suggest that the interaction
between these islands starts earlier than the maximum surface pressure achieved as indicated by point \( A \) in figure 2.5(b). After a small transition period, the monolayer reaches the “ultimate” collapse pressure. At this area, the islands of the condensed phase fill the interface completely. Compressing the monolayer further does not yield any increase in the surface pressure, but allows for more rearrangements at the interface. It is observed that the surface pressure measured here is kept constant by further compression and does not fall to lower values as suggested by other studies [65].

DPPC was chosen here for two reasons; it is the main component of lung surfactant and it can achieve very low surface tension (very high surface pressure) values upon compression. This is shown in the results of the collapse pressure values presented here which are in good agreement with values in the literature [14, 20]. Upon compression, DPPC monolayer forms flexible domains in the coexistence region of low density and condensed phases. The ability of DPPC to adjust to an area reduction is attributed to the change in the orientation of the hydrophobic tails of the DPPC molecule [102–104]. When the average area per molecule for the polar head decreases, the tilt angle of the tail also decreases and the orientation becomes more vertical. However, the large head group prevents the complete erection of the chains. These processes are responsible for some of the irregularities in the vicinity of the collapse area as shown in figure 2.6(b).

Comparing figures 2.5(a) and 2.6(a) the results confirm that the range of area per molecule over which the compression occurs is broader for DPPC than for octadecanol. Because of the higher flexibility of the domains, a DPPC monolayer may be compressed over a larger area per molecule range. This was attributed to the ordering of the DPPC tails towards vertical orientation which requires more energy, extending the range of area per molecule over which the compression occurs [66].

DPPG is a lipid found in lung surfactant where it represents 5-10% of the total phospholipid content. Unlike DPPC (the main lipid in lung surfactant), DPPG is ionic and forms charged monolayers on aqueous electrolyte subphases. The collapse pressure
depends on the nature of the subphase used. The values reported here are in good agreement with previous results, where DPPG was deposited on an air/water interface \[21\]. Other studies show a different collapse pressure value when DPPG was deposited onto subphases composed of solutions of sodium bicarbonate with varying NaCl salt concentrations \[22\].

Apart from the collapse pressure value, the DPPG isotherm has a similar shape to that of DPPC. However, figure 2.8(b) shows no signs of collapse prior to reaching the collapse pressure value. The change of the isotherm slope occurs at the collapse pressure as shown in figure 2.8(b). Further compression causes squeeze out from the DPPG monolayer. This squeezed out material organizes as aggregates in the subphase decreasing the surface pressure \[22\]. More compression will cause the surface pressure to increase again until another portion of the material is squeezed out. This would explain the irregularities in the isotherm in the vicinity of the collapse pressure as shown in figure 2.8(b). Studies using Brewster Angle Microscopy (BAM) and Atomic Force Microscopy (AFM) reveal that the squeezed out aggregates diffuse away from the monolayer at low ionic strength and remain in the subphase; at high ionic strength, they remain attached to the monolayer and reincorporate again into the monolayer when the film is expanded \[22\].

In conclusion, the use of a constrained sessile drop constellation (ADSA–CSD) as a film balance is illustrated. It was shown that it has certain advantages over conventional methods. It is a very simple setup and only small quantities of liquid are required. The ability to measure very low surface tension values, easier deposition procedure and leak-proof design makes the constrained sessile drop constellation a better choice than the pendant drop for studies of both soluble or insoluble monolayers.

The fact that ADSA–CSD measures the change of both surface area and surface tension with time allows a detailed study of the collapse region. Changes in surface area in the vicinity of the ultimate collapse pressure allow an estimate of the size of regions and the number of molecules that are being pushed into the subphase. Such information
Figure 2.9: The change of surface pressure with area per molecule of a constrained sessile drop of a DPPC monolayer for different runs.

is of specific interest for lung surfactant systems and cannot be obtained by a Langmuir film balance.

The results of compression isotherms for different systems show distinctive differences between the onset of collapse and the ultimate collapse pressure (ultimate strength) of these films. ADSA–CSD allows detailed study of this collapse region.

2.2.4 Results for Mixed Monolayers

Figure 2.9 shows the change of surface pressure with area per molecule for a DPPC monolayer at a compression speed of 0.19 cm²/min (19 Å²/molecule-min). The figure shows three different runs, illustrating reproducibility. The surface pressure starts at
approximately 5 mJ/m² and reaches about 70 mJ/m² as the film is compressed. A plateau is observed indicating the collapse of the monolayer at this ultimate collapse surface pressure (very low surface tension, approximately 2 mJ/m²). Further inspection shows that there is a slight change (not readily apparent in the figure) in the isotherm slope starting at pressures round 48 mJ/m², well below the ultimate collapse pressure. This point was denoted in the previous section as the onset of collapse.

The change of surface pressure with area per molecule of a constrained sessile drop of mixed DPPC/DPPG monolayers for different mixture ratios are compared in Figure 2.10 in the vicinity of the collapse pressure. The ratios studied here ranges from 100 to 50% DPPC (with 10% increments) in the mixture in addition to pure DPPG. It can be seen from the figure that for ratios 90:10 and 80:20, the DPPC/DPPG mixture can achieve a very high ultimate collapse pressure, similar to pure DPPC. When the ratio of DPPG in the mixture is increased, the ultimate collapse pressure is decreased slightly for the ratios 70:30 and 60:40. Upon further increase of DPPG (50% or more), the ultimate collapse pressure is decreased to approximately 60 mJ/m², similar to pure DPPG.

Every experiment for a specific mixture ratio was repeated at least 4 times. The reproducibility of the results shown in Figure 2.9 for the case of pure DPPC is not
Table 2.3: Ultimate collapse pressure (mJ/m$^2$) and film elasticity (mJ/m$^2$) at room temperature of 24°C from different runs of different DPPC:DPPG mixture ratios and the average value along with the standard deviation.

<table>
<thead>
<tr>
<th>DPPC:DPPG</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Run 4</th>
<th>Average</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Ultimate Collapse Pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100:0</td>
<td>70.7±0.03</td>
<td>70.6±0.07</td>
<td>70.6±0.14</td>
<td>70.7±0.04</td>
<td>70.6</td>
<td>0.02</td>
</tr>
<tr>
<td>90:10</td>
<td>70.5±0.11</td>
<td>70.4±0.25</td>
<td>70.5±0.24</td>
<td>70.2±0.13</td>
<td>70.4</td>
<td>0.05</td>
</tr>
<tr>
<td>80:20</td>
<td>70.9±0.24</td>
<td>70.5±0.29</td>
<td>70.8±0.26</td>
<td>70.7±0.21</td>
<td>70.7</td>
<td>0.07</td>
</tr>
<tr>
<td>70:30</td>
<td>68.7±0.23</td>
<td>69.0±0.41</td>
<td>68.1±0.44</td>
<td>67.4±0.82</td>
<td>68.3</td>
<td>0.28</td>
</tr>
<tr>
<td>60:40</td>
<td>68.6±0.80</td>
<td>69.9±0.61</td>
<td>70.0±0.36</td>
<td>67.2±0.58</td>
<td>68.9</td>
<td>0.50</td>
</tr>
<tr>
<td>50:50</td>
<td>60.8±1.39</td>
<td>60.4±1.10</td>
<td>60.2±0.99</td>
<td>61.0±1.90</td>
<td>60.6</td>
<td>0.15</td>
</tr>
<tr>
<td>0:100</td>
<td>60.8±1.12</td>
<td>59.6±1.68</td>
<td>60.0±1.42</td>
<td>59.2±1.82</td>
<td>59.9</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td><strong>Film elasticity at π = 50 mJ/m$^2$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100:0</td>
<td>227.5±1.4</td>
<td>236.4±2.0</td>
<td>225.2±0.7</td>
<td>221.5±1.7</td>
<td>227.6</td>
<td>2.4</td>
</tr>
<tr>
<td>90:10</td>
<td>243.7±1.0</td>
<td>209.4±5.3</td>
<td>257.5±4.4</td>
<td>255.7±4.0</td>
<td>241.6</td>
<td>8.4</td>
</tr>
<tr>
<td>80:20</td>
<td>212.4±1.5</td>
<td>214.1±1.6</td>
<td>203.5±2.7</td>
<td>207.8±1.0</td>
<td>209.4</td>
<td>1.8</td>
</tr>
<tr>
<td>70:30</td>
<td>209.3±1.7</td>
<td>224.8±2.8</td>
<td>198.9±1.1</td>
<td>263.0±2.1</td>
<td>224.0</td>
<td>10.6</td>
</tr>
<tr>
<td>60:40</td>
<td>191.9±1.7</td>
<td>214.8±1.6</td>
<td>208.8±1.0</td>
<td>224.7±2.3</td>
<td>210.1</td>
<td>5.2</td>
</tr>
<tr>
<td>50:50</td>
<td>199.2±1.4</td>
<td>250.4±3.1</td>
<td>168.5±1.3</td>
<td>227.3±1.2</td>
<td>211.3</td>
<td>13.4</td>
</tr>
<tr>
<td>0:100</td>
<td>774.6±31.8</td>
<td>755.3±38.2</td>
<td>717.8±18.7</td>
<td>736.4±59.9</td>
<td>746.0</td>
<td>9.2</td>
</tr>
</tbody>
</table>
always guaranteed. A summary of calculated ultimate collapse pressure for different mixture ratios is shown in Table 2.3 along with an average value and standard deviation. To illustrate the reproducibility of the results for different mixture ratios, Table 2.3 shows the ultimate collapse pressure values obtained for all runs of every mixture ratio. The table shows that adding a limited amount of DPPG (up to 20%) to DPPC monolayer does not alter the ultimate collapse pressure. Increasing the DPPG content in the mixture (30% or 40%) shows a transition region, where the ultimate collapse pressure is decreased to values ranging between 66 and 70 mJ/m\(^2\). The variations of values obtained for the ultimate collapse pressure in this regions are not small, indicating a low reproducibility. However, further increase of DPPG content in the mixture (50% and beyond) causes a sharp decrease in the ultimate collapse pressure to values close to the pure DPPG. It is noted that the reproducibility in this region is much better than that of the transition region.

Further insight into the differences between the mixture ratios can be gained by calculating the surface dilatational elasticity from the measured isotherms. For reason of comparison, elasticity values are calculated at the same surface pressure, i.e. 50 mJ/m\(^2\), for all the measured isotherms. This value is chosen for two reasons; first, it is below the ultimate collapse pressure for the isotherms in this study. Second, this value is close to the equilibrium surface pressure for lung surfactant systems, thus eliminating any effects of relaxation or adsorption that could alter the calculated value of film elasticity. A summary of calculated film elasticity values for different mixture ratios is shown in Table 2.3 along with an average value and standard deviation. It can be seen that for all ratios studied here, the elasticity values are around 220 mJ/m\(^2\), very close to pure DPPC. Increasing the DPPG content in the mixture does not increase the film elasticity. However, the pure DPPG film has a much higher elasticity of approximately 750 mJ/m\(^2\), i.e. a better film property.

Thus far, experiments were performed by only compressing the film once and record-
Figure 2.11: The change of surface pressure with area per molecule on successive compressions and expansions of a constrained sessile drop of mixed DPPC/DPPG monolayers for different mixture ratios: (a) 100:0; (b) 90:10; (c) 80:20.
ing the change of surface pressure. A different set of experiments were performed, where after the full compression of the monolayer (different mixture ratios), the monolayer was then expanded and compressed again. This compression/expansion cycle was then repeated, i.e. 3 compression/expansion cycles for each mixture ratio. Figure 2.11 shows the different compression/expansion cycles for three different mixture ratios, i.e. pure DPPC, 90:10 and 80:20 DPPC/DPPG. These ratios were selected because all of them reach the same high ultimate collapse pressure shown in Table 2.3. The second compression of pure DPPC was slightly shifted to the left compared to the first compression as shown in Figure 2.11(a). This indicated that recompression of pure DPPC causes a small loss of molecules between the first and second compression. For the case of 10% DPPG (Figure 2.11(b)), after the first compression, the shape of the compression/expansion isotherms is very similar to the pure DPPC isotherm. This suggests that during the first compression, most of the DPPG was ejected from the interface leaving a pure DPPC monolayer for the following compression/expansion cycles. However, increasing the DPPG content to 20% (Figure 2.11(c)) shows a change in the shape of the cycle. After the first compression, the shape of the subsequent compressions did not change. Every compression in this case shows a slight shift to the left. It is noted that the shape here is different from that of pure DPPC for all 4 compressions.

2.2.5 Discussion for Mixed Monolayers

2.2.5.1 Ultimate Collapse Pressure of Mixed Monolayers

As the main component of lung surfactant, DPPC is known to achieve very low surface tension (very high surface pressure) values upon compression. This is shown in the results of the collapse pressure values for pure DPPC presented here in Figure 2.9 and Table 2.3. These results are in good agreement with values in the literature [14, 20, 88]. Unlike DPPC, the collapse pressure of DPPG depends on the nature of the subphase used. The
values for pure DPPG reported here in Table 2.3 are in good agreement with previous results, where DPPG was deposited on an air/water interface [21, 70, 88].

Results presented in Table 2.3 show that adding a limited amount of DPPG (up to 20%) to DPPC does not alter the ability of the monolayer to reach a very high ultimate collapse pressure. With further increase of the amount of DPPG in the mixture, the ultimate collapse pressure goes through a transition region followed by a decrease in the ultimate collapse pressure to values close to that of the pure DPPG (for ratios of 50% and beyond). These results suggest that an increased amount of DPPG (beyond 30%) would adversely affect the ability of the mixture to reach high surface pressure (low surface tension) values.

2.2.5.2 Film Elasticity of Mixed Monolayers

In addition to the high collapse pressure, a good monolayer would also have a high elasticity value. The elasticity can be calculated from the slope of a $\pi - A$ isotherm as shown in Table 2.3. The monolayer elasticity is a measure of the film resistance (change in the film surface pressure) to a change in the area per molecule. The results show that the monolayer elasticity calculated at 50 mJ/m$^2$ surface pressure is around 220 mJ/m$^2$ for pure DPPC, and around 750 mJ/m$^2$ for pure DPPG. The value for pure DPPC is in good agreement with previous results [105], and the value for pure DPPG is in agreement with values calculated from previously measured isotherms [88]. Some of the calculated elasticity values measured from previously measured isotherms [70, 106] are somewhat lower (between 350 and 600 mJ/m$^2$) than the value measured here, but still higher than that of pure DPPC. However, the resolution in the current data in the vicinity of 50 mJ/m$^2$ surface pressure is much better than in other techniques. As elasticity is a function of compression rate, it should be kept in mind that the compression rate for all experiments was fixed at 0.19 cm$^2$/min, as indicated earlier. The relatively large error for DPPG elasticity in Table 2.3 is due to the fact that the experimental isotherms are very
steep, i.e. approaching a slope of infinity. All DPPC/DPPG mixture ratios studied here (90 to 50% DPPC) have elasticity values very close to pure DPPC. It is clear that pure DPPG films have better elasticity than DPPC films. However, increasing the DPPG content in the mixture does not improve the film elasticity.

2.2.5.3 Repeated Compression Cycles

In the first set of experiments, after spreading, the monolayer was continuously compressed until collapse, at which point the experiment ended. In the second set of experiments, the monolayer was spread, then compressed until collapse occurred. The monolayer was then expanded and compressed again. Three compression/expansion cycles were performed for every experiment. The difference between the first and the following compressions usually gives an indication of the number of molecules lost between compressions. Three DPPC/DPPG mixture ratios are compared in Figure 2.11. For the case of pure DPPC (Figure 2.11(a)), the results suggest that some molecules were not able to reincorporate at the interface after the first compression. The successive compressions did not show significant change. For the case of 10% DPPG in the mixture, the shape of the compression/expansion cycles after the first compression is essentially the same as that of pure DPPC as shown in Figure 2.11(b). This suggests that the 10% DPPG content was squeezed out from the interface during the first compression and was not able to reincorporate back. The interface after the first compression is probably composed of DPPC only. On the other hand, increasing the DPPG content to 20% (Figure 2.11(c)) shows a change in the shape of the compression/expansion cycles. Every compression in this case shows a slight shift to the left, indicating a continuous ejection of molecules. It is noted that the shape here is different from that of pure DPPC for all compressions. This suggest that, at this mixture ratio, DPPG is still present at the interface at the high ultimate collapse pressure.
2.2.5.4 Implications for Lung Surfactant Development

Conceptually, it is possible to define qualitatively a set of surface properties that are necessary for the respiratory function of lung surfactants. These surface properties include the ability to adsorb to the air/water interface, the ability to reach very low surface tension values during dynamic cycling, the ability to reach such low surface tension values with minimum amount of area reduction (high elasticity), and the ability to respread at the interface during expansion \[1\]. No single component of the complex compound of lung surfactant has all these necessary surface properties. However, different components interact to achieve the required system behaviour. As shown in the literature, and reported here as well, DPPC alone (zwitterionic phospholipid) can reach extremely high surface pressures (low surface tensions below 2 mJ/m\(^2\)). However, DPPC adsorbs very slowly to the air/water interface \[1, 36\], has a relatively low elasticity compared to DPPG as shown in Table 2.3, and respreads poorly in compression/expansion cycles as shown in Figure 2.11(a). Fluid phospholipids, neutral lipids and surfactant proteins (SP-A, SP-B, and SP-C) cannot reach high surface pressures but have a significant effect on improving adsorption and respreading \[61, 62, 107\].

The presence of anionic phospholipids, e.g. DPPG, in lung surfactant is well established; however, their quantitative contribution to surface activity is less clear. Results presented here suggest that an increased content of DPPG will adversely affect the surface tension lowering ability of DPPC. However, it is also shown that DPPG has better film elasticity and respreading properties in compression/expansion cycles. It is widely believed that their association with one or more surfactant proteins may make a contribution to the surface activity \[96, 100, 108, 115\]. As mentioned before a DPPC/PG mixture has been repeatedly used to mimic natural lung surfactant \[94, 95\], and has also been used as the main component of many clinical synthetic exogenous lung surfactants, e.g. ALEC \[79\], Surfaxin \[82\], and Venticute \[85\].

In conclusion, results here suggest that DPPG improves the respreading properties
of the mixture and may improve the film elasticity. The only drawback of increasing the content of DPPG is the adverse effect on the ultimate collapse pressure. However, the low collapse pressure of DPPG is limited only to pure water as subphase. Other studies showed that DPPG can in fact achieve very high collapse pressure values (similar to pure DPPC) when deposited onto subphases composed of solutions of sodium bicarbonate mixed with NaCl [22]. This leads to the expectation that DPPG may play an important role in the future of lung surfactant development.

### 2.3 Double Injection Capability

Lung surfactant inhibition or inactivation is a phenomenon caused by processes that degrade the surface activity of the surfactant [1]. Direct interaction with endogenous inhibitors is a main reason for such phenomena. Some inhibitors can impair the adsorption and the surface tension lowering abilities of the surfactant. Examples of such inhibitors include plasma and blood proteins (such as albumin, fibrinogen, and hemoglobin) [116–122], unsaturated cell membrane phospholipids [121, 123], lysophospholipids [34, 124], fluid free fatty acids (such as oleic acid) [35, 125], meconium [126, 127], and cholesterol when present at high concentrations [128, 129]. Other inhibitors, usually released during lung inflammation or injury, can chemically react with and degrade the functional components of the surfactant. Examples include lytic enzymes (proteases and phospholipases) [33, 130] and reactive oxidant species [131, 132].

The surface activity of lung surfactants can be assessed with various methods as shown in Chapter [1]. Most of the methods suffer from one or more of the following limitations: inability to perform fast cycling, film leakage, uncontrolled environmental conditions, inaccurate calculation of low surface tension values, inoperability at the high surfactant concentrations. These limitations must be removed in the in vitro study of lung surfactants that should mimic the conditions of normal breathing. ADSA–CSD
is believed to be free of all restrictions and limitations of other conventional methods mentioned before. However, it is not possible yet to use ADSA–CSD as a penetration film balance that would allow the access to the interface from the liquid side.

The purpose of this section is to present a modified design for ADSA–CSD featuring a secondary injection system facilitating access to the interface from the liquid side. The study of the effect of a specific inhibitor on a preformed lung surfactant film is a good illustration of this capability. After forming a sessile drop of a basic surfactant preparation, the bulk phase can be exchanged and different inhibitors such as serum, albumin, fibrinogen, and cholesterol can be introduced. Results are compared, below, with the case where the inhibitor and the surfactant preparation are premixed and co-adsorb. This modified design can be used to evaluate lung surfactant inhibition, resistance and reversal under completely controlled physiological conditions. The modified design can also be used for bulk liquid sampling and on-site mixing.

2.3.1 Experimental Details

2.3.1.1 Materials and Methodology

The lung surfactant used, Bovine Lipid Extract Surfactant (BLES), was provided by BLES Biochemicals Inc. (London, Ontario, Canada) at a concentration of 27 mg/ml and was used without further purification. BLES was divided into 1 ml glass vials in Ar atmosphere and stored at -20 °C. On the day of the experiment, one vial was maintained at 37.5 °C water bath for one hour before diluting it to 2.0 mg/ml by a salt solution of 0.6% NaCl and 1.5 mM CaCl₂. The pH value of the diluted BLES preparation ranged from 5 to 6.

A potential lung surfactant additive, protasan (UP CL 213, chitosan chloride, deacetylation 75-90%), was purchased from NovaMatrix (Norway). More details about protasan can be found elsewhere [133, 134]. Protasan was dissolved in the NaCl/CaCl₂ salt solu-
tion on the day of the experiment and then diluted to the final concentration (ranging from 0.05 to 0.50 mg/ml according to the required preparation) and mixed with 2.0 mg/ml BLES suspension. The pH of these BLES-protasan mixtures ranged from 5 to 5.5.

Here, a preparation of BLES and protasan is chosen as the basic preparation, i.e. a basis for comparison. Through accumulated experience in the Laboratory of Applied Surface Thermodynamics, it was observed that the minimum surface tension of BLES alone, without any additives, varies significantly from batch to batch [91], at least at low BLES concentrations. The minimum surface tension, i.e. the lowest surface tension obtainable upon 20% area reduction, varied from 5.5 mJ/m$^2$ to 20.4 mJ/m$^2$ in 100% relative humidity [91]. Parenthetically, it is noted that typically these minimum surface tensions are much lower at non-physiological lower humidities. Therefore, in order to study whether a certain inhibitor has the capability of raising the surface tension above the physiologically interesting range of 1 to 5 mJ/m$^2$, we need a standard enhancing additive to guarantee an initial low surface tension, prior to any challenge of the surfactant film by an inhibitor. The choice of additive here is protasan (chitosan chloride). A similar procedure was described previously for the case of chitosan as a surfactant additive [91].

Serum (bovine, cat. B8655), albumin (from bovine serum, fatty acid free, globulin free, $\geq$99%, cat. A0281), fibrinogen (from bovine plasma, type I-S, 65-85% protein, cat. F8630), and cholesterol ($\geq$99%, cat. C8667) were purchased from Sigma-Aldrich Co. Serum was diluted in the NaCl/CaCl$_2$ salt solution to a final concentration, ranging from 0.01 to 0.30 ml/ml according to the required preparation. Albumin was dissolved in the NaCl/CaCl$_2$ salt solution to a concentration of 40.0 mg/ml. Fibrinogen was dissolved in a salt solution of 0.85% NaCl and 1.5 mM CaCl$_2$ and adjusted to a concentration of 3.0 mg/ml. Cholesterol was dissolved in 10 mg/ml ethanol and diluted to a final concentration of 0.284 mg/ml. The pH value of the diluted serum preparations was near 7, while the pH value of the other inhibitor preparations ranged from 5 to 6.
2.3.1.2 Experimental Procedure

The design for a modified ADSA–CSD including a secondary injection was developed and manufactured. The details of the design and operation of the original ADSA–CSD were described elsewhere [25, 46, 48]. A schematic diagram of the new design is shown in figure 2.12 and a detailed engineering drawing is shown in figure 2.13. The modified design features a capillary needle fitted concentrically in the opening of the main pedestal (holder) to facilitate secondary liquid injection. The capillary needle is connected to a separate syringe with a dedicated motor equipped with a controller.

The details of the modified ADSA–CSD are as follows: the primary fluid (lung surfactant preparation) is delivered from the first syringe to form a sessile drop on a circular horizontal stainless steel pedestal. The secondary fluid (inhibitor) is injected from the second syringe into the preformed sessile drop through a capillary needle. The base of the pedestal is designed to accommodate a reservoir, which is used to ensure a constant
Figure 2.13: Detailed engineering drawing of double injection ADSA–CSD.
temperature.

During the experiment, the setup is enclosed in an environmental control chamber that facilitates the control of gas composition and temperature. The humidity inside the chamber was kept constant at 100% relative humidity at 37 °C. Two stepping motors (controller 18705/6, Oriel Instruments, Stratford, CT) were used, one for each fluid, to facilitate the fluid volume injection/extraction and the compression/expansion during the dynamic cycling part of the experiment. A CCD camera (model 4815-5000, Cohu Corp., Poway, CA) mounted on a microscope (type 400076, Wild Heerburgg, Switzerland) was used to acquire images throughout the experiment at a rate of 20 images per second. The images were digitized using a digital video frame grabber (Snapper-8, Active Silicon Ltd., Iver, UK) and stored in a workstation (SunBlade 1500, Sun Microsystems, Santa Clara, CA) for further analysis by ADSA.

The experimental procedure for a double injection experiment is as follows: First, the capillary needle is filled with the secondary fluid (inhibitor). Then, the primary fluid (lung surfactant) is used to form a sessile drop on the pedestal. The surface tension is tracked until the film reaches the equilibrium surface tension (within 180 seconds). Thereafter, dynamic cycling is performed by successive compression/expansion of the drop. This is normally done through 20 cycles with a periodicity of 3 seconds per cycle and a compression ratio (percentage of surface area reduction) of 20% to simulate normal human breathing conditions [1, 25]. Unless stated otherwise, compression conditions in all results in this work are the same (20% surface area reduction at 3 s/cycle periodicity) in humid air (100% R.H.).

At this point, the volume replacement procedure begins. The secondary fluid is injected continuously through the inner capillary needle. During the injection, the primary fluid is simultaneously and continuously withdrawn using the other syringe and motor controller. The volume exchanged is 5 times the drop volume to ensure a complete bulk replacement of the primary fluid by the secondary fluid [60]. This minimum exchange
volume depends on flow dynamics as shown earlier [135]. To corroborate the recommendations of Cabrerizo-Vilchez et al. [60], the volume exchange process was tested using two miscible liquids with the same density but with different interfacial tensions, namely dimethylsulfoxide (DMSO) and 1-chlorobenzene (CB). It was found that complete bulk replacement can be ensured after only 2 volumes exchange for the specific dynamic flow condition used here.

The volume replacement is performed slowly (120 seconds) to avoid film disturbance. After completing this step, the drop is left for 180 seconds to reach equilibrium again. Finally, the dynamic cycling part is repeated (20 cycles, 3 seconds per cycle, 20% compression). The results from the two dynamic cycling parts (before and after injection) are further analyzed and compared to evaluate the effect of injecting the secondary fluid (usually an inhibitor) under a preformed film of the primary fluid (the basic preparation).

One of the main parameters that reflects the effect of injection is the minimum surface tension, \( \gamma_{min} \), obtained at the end of compression in each cycle during the two dynamic cycling parts. The reported values here are those averaged over the 20 cycles during a specific dynamic part along with the standard deviation. The measured value of minimum surface tension depends on the compression conditions. Another important parameter is the dilatational elasticity as defined in equation 2.4. The elasticity can be calculated in a similar fashion to equation 2.4 but written in terms of surface tension [25, 45, 47, 62, 107, 136].

\[
\epsilon = \left| \frac{d\gamma}{d\ln A} \right| \quad (2.5)
\]

where \( \gamma \) is the surface tension of the film at any time, and \( A \) is the drop surface area at any time. The change of surface tension with the relative surface area during the compression part of every cycle is fitted to a fourth order polynomial and the slope is evaluated at the midpoint of the compression [91]. The calculated slope and the value of the relative surface area are used to calculate the dilatational elasticity according to equation 2.5.
Figure 2.14: Change in surface tension with time and with relative surface area during dynamic cycling: (a) 2.0 mg/ml BLES and 0.2 mg/ml protasan (basic preparation); (b) After replacing the bulk phase with salt solution. All compressions/expansions are performed at 3 s/cycle periodicity in humid air (100% R.H.).

2.3.2 Results

Figure 2.14 shows a solvent exchange experiment. The change of surface tension, $\gamma$, with time during the first few cycles in the dynamic cycling of a basic preparation of 2.0 mg/ml BLES and 0.2 mg/ml protasan is shown in figure 2.14(a). The figure also shows the change of surface tension with relative surface area, $A_r$, for one of the cycles (the fifth cycle). Figure 2.14(b) shows the surface tension response for the same compression conditions but after replacing the bulk with salt solution only (bulk wash-out). The minimum surface tension after bulk replacement did not change. Both cases have the same $\gamma - A_r$ response with similar elasticity. The consistency of the surface activity after bulk replacement suggests effectively irreversible adsorption. This is consistent with the
spreading of lung surfactant vesicles described in the literature [137–139].

Figure 2.15(a) shows the change of surface tension, $\gamma$, with time during the first few cycles in the dynamic cycling of a basic preparation of 2.0 mg/ml BLES and 0.2 mg/ml protasan. The rest of the 20 cycles did not show a significant difference from the first few. The figure also shows the change of surface tension with relative surface area, $A_r$, for one of the cycles (the fifth cycle). The open circles show the change during the compression part of the cycle, while the solid circles give the surface tension during the expansion part. The minimum surface tension is $2.9 \pm 0.3$ mJ/m$^2$. Figure 2.15(b) shows the surface tension response for the same compression conditions but after injecting 0.15 ml/ml serum into the bulk of the basic preparation. The minimum surface tension after the injection is $3.5 \pm 0.6$ mJ/m$^2$. Comparing figures 2.15(a) and 2.15(b) show that injecting serum under a preformed film of BLES and protasan has little effect on the minimum surface tension. However, the $\gamma - A_r$ isotherms show a significant change in the cycle hysteresis and also in the slope of the compression part of the cycle.

Figure 2.15(c) shows the change of surface tension with time and with relative surface area for the mixture of the inhibitor (0.15 ml/ml serum) and the basic preparation (2.0 mg/ml BLES and 0.2 mg/ml protasan) in a separate single injection experiment. In this experiment, the mixture is used to form a sessile drop on the pedestal. Then, the surface tension is tracked until the film reaches the equilibrium surface tension (within 180 seconds). After that, dynamic cycling is performed under standard conditions [25]. The minimum surface tension for the mixture is $15.6 \pm 0.3$ mJ/m$^2$, which differs significantly from the value achieved after the injection in the double injection experiment. The $\gamma - A_r$ isotherms show that the cycle hysteresis and the slope of the compression is also different from figures 2.15(a) and 2.15(b).

The choice of the basic preparation in that experiment needs further comment. To determine a suitable concentration of protasan to be added to BLES, different concentrations were tested and the minimum surface tension obtained for two different BLES
Figure 2.15: Change in surface tension with time and with relative surface area during dynamic cycling: (a) 2.0 mg/ml BLES and 0.2 mg/ml protasan (basic preparation); (b) After injecting 0.15 ml/ml serum into the bulk phase; (c) 2.0 mg/ml BLES and 0.2 mg/ml protasan and 0.15 ml/ml serum mixed in a separate single injection experiment. All compressions/expansions are performed at 3 s/cycle periodicity in humid air (100% R.H.).
Figure 2.16: Minimum surface tension (end of compression) as a function of protasan concentration for different batches of BLES (2.0 mg/ml). All compressions (20%) are performed at 3 s/cycle periodicity in humid air (100% R.H.).
batches (2.0 mg/ml) was plotted in figure 2.16. These values were obtained during the dynamic cycling of single injection experiments. It is clear that the minimum surface tension of BLES alone, without any protasan, differs in these two batches from 5.5 mJ/m$^2$ to 11.0 mJ/m$^2$. It is also clear that adding a small concentration of protasan reduces the minimum surface tension of both BLES batches; this is similar to the effect of chitosan on different BLES batches [91]. In view of this figure, a preparation of 2.0 mg/ml BLES and 0.2 mg/ml protasan is used as the basic preparation.

Figure 2.17 (a) shows the change of surface tension, $\gamma$, with time during the first few cycles in the dynamic cycling after injecting a higher concentration of serum, i.e. 0.30 ml/ml, into the bulk of the basic preparation (2.0 mg/ml BLES and 0.2 mg/ml protasan).
Chapter 2. ADSA-CSD as a Micro Film Balance

Figure 2.18: Change in surface tension with time and with relative surface area during dynamic cycling: (a) After injecting 40 mg/ml albumin into the bulk phase of 2.0 mg/ml BLES and 0.2 mg/ml protasan; (b) 2.0 mg/ml BLES and 0.2 mg/ml protasan and 40 mg/ml albumin mixed in a separate single injection experiment. All compressions/expansions are performed at 3 s/cycle periodicity in humid air (100% R.H.).

The figure also shows the change of surface tension with relative surface area, $A_r$, for one of the cycles. Figure 2.17(b) shows the change of surface tension with time and with relative surface area for the mixture of the inhibitor (0.30 ml/ml serum) and the basic preparation (2.0 mg/ml BLES and 0.2 mg/ml protasan) in a separate single injection experiment. Figures 2.18, 2.19, and 2.20 show similar results for different inhibitors: 40.0 mg/ml albumin, 3.0 mg/ml fibrinogen, and 0.28 mg/ml cholesterol, respectively. Results for injecting an inhibitor under a preformed film of the basic preparation and mixing the inhibitor with the preparation (letting them adsorb together to form a film) show a similar pattern for a wide range of inhibitors.

Results for albumin (figure 2.18) show that mixing with the basic preparation causes
Figure 2.19: Change in surface tension with time and with relative surface area during dynamic cycling: (a) After injecting 3.0 mg/ml fibrinogen into the bulk phase of 2.0 mg/ml BLES and 0.2 mg/ml protasan; (b) 2.0 mg/ml BLES and 0.2 mg/ml protasan and 3.0 mg/ml fibrinogen mixed in a separate single injection experiment. All compressions/expansions are performed at 3 s/cycle periodicity in humid air (100% R.H.).

an increase in the minimum surface tension and a decrease in film elasticity during compression. On the other hand, the injection of albumin under a preformed surfactant film did not change the minimum surface tension. However, injecting albumin caused an increase in the film elasticity during compression, leading to a minimum surface tension at a lower compression ratio, i.e. at approximately 12%, compared to the standard 20%. Further compression caused film collapse (no further decrease in surface tension) leading to film hysteresis as shown in figure 2.18(a).

Results for fibrinogen (figure 2.19) show a dramatic increase in the minimum surface tension and a corresponding decrease in film elasticity during compression when fibrinogen was mixed with the basic preparation. However, when fibrinogen is injected under
Figure 2.20: Change in surface tension with time and with relative surface area during dynamic cycling: (a) After injecting 0.284 mg/ml cholesterol into the bulk phase of 2.0 mg/ml BLES and 0.2 mg/ml protasan; (b) 2.0 mg/ml BLES and 0.2 mg/ml protasan and 0.284 mg/ml cholesterol mixed in a separate single injection experiment. All compressions/expansions are performed at 3 s/cycle periodicity in humid air (100% R.H.).

a preformed film, the minimum surface tension in the first cycle was very close to that of the basic preparation. Starting with the second cycle, the minimum surface tension increased slightly and stayed constant during the rest of the 20 cycles. In this case, it can be seen that the film elasticity during compression has decreased as well.

Results for cholesterol (figure 2.20) show an increase in the minimum surface tension and a decrease in film elasticity during compression when cholesterol was mixed with the basic preparation. On the other hand, when injected under a preformed surfactant film, cholesterol does not tend to change the minimum surface tension, and in fact tends to improve the film elasticity during compression. This can be seen from the increased slope of the $\gamma - A_r$ isotherm shown in figure 2.20(a) when compared to the corresponding
Figure 2.21: Comparison of minimum surface tension for different inhibitors injected or mixed into a basic preparation of 2.0 mg/ml BLES and 0.2 mg/ml protasan. The dashed line shows the minimum surface tension of the basic preparation. All compressions (20%) are performed at 3 s/cycle periodicity in humid air (100% R.H.).

A comparison of minimum surface tension obtained during dynamic cycling for different inhibitors injected or mixed into the basic preparation of 2.0 mg/ml BLES and 0.2 mg/ml protasan is shown in figure 2.21. For all the inhibitors tested here, the minimum surface tension of the basic preparation (before injection) did not change significantly after injecting the inhibitor. However, when the same inhibitors are mixed with the basic preparation and adsorb together to form a film, the minimum surface tension is significantly higher as shown in figure 2.21.

The corresponding comparison for the dilatational elasticity, $\epsilon$, obtained during dynamic cycling for different inhibitors injected as well as mixed into the basic preparation
Figure 2.22: Comparison of elasticity of compression for different inhibitors injected or mixed into a basic preparation of 2.0 mg/ml BLES and 0.2 mg/ml protasan. The dashed line shows the elasticity of compression of the basic preparation. All compressions (20%) are performed at 3 s/cycle periodicity in humid air (100% R.H.).
of 2.0 mg/ml BLES and 0.2 mg/ml protasan is shown in figure 2.22. The trends here are similar to the comparison of the minimum surface tension. For the cases of serum and albumin, the elasticity of the film of the basic preparation did not change significantly after injecting the inhibitor. However, for the case of fibrinogen, the injection caused the elasticity to decrease to reach a value close to that obtained from the mixture. Interestingly, cholesterol apparently increased the elasticity (improved the film properties) after being injected under the preformed film of the basic preparation.

So far, only the effect of injecting an inhibitor under a preformed film of an active basic preparation has been studied. Another important aspect that can be studied using this new technique is inhibition reversal, i.e. the ability to restore the normal surface activity of an inhibited lung surfactant film. An experiment illustrating this concept is
shown in figure 2.23. In this experiment, a film was originally formed using 0.01 ml/ml serum only (as the basic preparation in this case). Changes of surface tension with time and with relative surface area during the dynamic cycling are shown in figure 2.23(a); the minimum surface tension obtained in this case is 38.0 ±0.2 mJ/m². A preparation of 2.0 mg/ml BLES and 0.2 mg/ml protasan is subsequently injected into the preformed film of serum, and the change of surface tension with time during the dynamic cycling (after the injection) is shown in figure 2.23(b). This figure shows 12 cycles out of a total of 20 cycles performed, the rest of the cycles did not show significant further changes. The change of surface tension with relative surface area is shown for the twelfth cycle. The minimum surface tension obtained after 12 cycles is 5.7±0.6 mJ/m². This effectively shows that a film of dilute serum can be replaced at least partially by a preparation of BLES and protasan.

2.3.3 Discussion

2.3.3.1 Methodology Evaluation

In order to manipulate and intervene with a lung surfactant film in vitro, it is desirable to have access to the film from both the liquid and the air side. In ADSA–CSD, access from the air side is quite easy, as shown in Section 2.2. A deposition procedure to spread films on the interface was developed and used. But access from the liquid side is also possible and the necessary development has been described above. As the present development does not affect any core parts of ADSA–CSD, the results have the same reliability as those of previous studies [25, 48, 76, 88, 91]. The vehicle chosen here to illustrate capabilities of bulk substrate manipulation is the resistance of existing lung surfactant films against inhibitors and, briefly, to document the possibility of reversing inhibition, i.e. the removal of inhibitors from the surface film.

A key feature of ADSA–CSD is the ability to use relevant physiological conditions in
terms of compression rates and environmental control (contamination, humidity, pressure and temperature). Other conventional methods also have some of these features, but typically not all. For example, a hypophase exchange system was used in conjunction with a Pulsating Bubble Surfactometer to study the effect of albumin as an inhibitor on a preformed lung surfactant film \[31\]. However, film leakage from the bubble surface into the capillary tube at low surface tension values is a common complication \[36\]. Other limitations are the inaccurate calculation of low surface tension values due to spherical shape assumption \[27\], and the difficulty of controlling the environmental conditions.

### 2.3.3.2 Inhibition Study: Effect of Film Formation

The leakage of plasma and blood proteins into the alveolar spaces is a common cause of severe lung injury. The results presented here show a distinctive difference between the inhibition of a specific inhibitor when mixed with and when injected under a preformed surfactant film. Generally, all the inhibitors studied here (serum, albumin, fibrinogen, and cholesterol) were not able to penetrate the film or affect the lowering surface tension ability of the chosen basic preparation film (BLES and protasan), while all of them can alter the surface activity when mixed with the preparation. Figure 2.21 compares the minimum surface tension achieved in both cases for every inhibitor. On the other hand, changes in the elasticity of the film during compression (i.e. the inverse of film compressibility) gives useful insight into the effect of every inhibitor. Some of the inhibitors studied here have altered the film compressibility without showing any changes in the minimum surface tension measured during the dynamic cycling. Detailed discussion for every inhibitor is given below.

Two concentrations of serum were studied here, 0.15 and 0.30 ml/ml. They show a similar pattern when mixed with the basic preparation (BLES and protasan). The minimum surface tension values measured for both concentrations was significantly higher and the elasticity of film compression was much lower than that for the basic preparation
alone. On the other hand, when the bulk phase of the basic preparation was replaced with serum (under a preformed film), the minimum surface tension did not change significantly. It can be seen from figures 2.15(b) and 2.17(a) that the first cycle after the bulk replacement can go to a very low surface tension value (indicating a good film at this point). Starting with the second cycle, the minimum surface tension increased slightly. Comparison of the $\gamma - A_r$ isotherms in the same two figures with figure 2.15(a) suggests that having serum under a preformed lung surfactant film does not change the minimum surface tension but it does increase the hysteresis as shown in the figures. Obviously, this increase in film hysteresis must be due to change in film properties other than the minimum surface tension. Such properties include the film elasticity during the compression part as shown in figure 2.22. Another important property is the film relaxation at the end of the compression, where further decrease in film area causes an increase in the surface tension. It is apparent that the characterization of inhibition mechanisms will require other surface properties in addition to the minimum surface tension; this will be discussed further in Chapter 3.

Results obtained here from premixing albumin and BLES shows the inhibition of the lung surfactant when it is to co-adsorb with albumin as shown in figure 2.18(b). The minimum surface tension was increased and the elasticity was decreased. However, for the case of introducing albumin under a preformed film of lung surfactant, results here suggest that the dynamic surface tension lowering ability of the film was not altered. Similar results were obtained before for a lower range of albumin concentrations (3 to 6 mg/ml) than used here using the hypophase exchange system with PBS [34]. However, since we have here complete information about the change of surface tension and area during the cycling, we are able to report that the elasticity of the film has changed as shown in figure 2.18(a). In this figure, the minimum surface tension was reached at a lower degree of area reduction. This suggests a slight increase in the film elasticity in this case.
Results obtained here suggest that fibrinogen is more potent than other inhibitors when mixed with lung surfactant preparations as shown in figure 2.21, causing a very significant increase in the minimum surface tension during the dynamic cycling. The severe inhibition effect of fibrinogen has been reported in previous studies [116, 117, 120, 122, 140]. However, results show that injecting fibrinogen under a preformed film of lung surfactant did not significantly change the minimum surface tension. As shown in figure 2.19(a), the first cycle after the bulk replacement can go to a very low surface tension value, but the minimum surface tension increased slightly starting from the second cycle. It is important to note that fibrinogen reduces the film elasticity much more severely than other inhibitors when injected under a preformed surfactant film as shown in figure 2.22.

Similar to the other inhibitors, cholesterol increases the minimum surface tension when mixed with lung surfactant preparation. Results show that the effect of the same concentration of cholesterol is different for the case of introducing the cholesterol to the subphase of a preformed film versus premixing with the lung surfactant preparation prior to film formation. When injected under a preformed film, cholesterol did not significantly change the minimum surface tension and it actually improved the film elasticity as shown in figures 2.21 and 2.22. Cholesterol, which is removed from complete lung surfactant in the preparation of BLES, is one of the main neutral lipids in lung surfactant with a concentration of around 10% of the surfactant lipids [141, 142]. Until recently, lung surfactant inhibition has been attributed to factors other than cholesterol. However, it was reported that elevated levels of cholesterol have the ability to impair and inhibit lung surfactant, affecting its ability of dynamic surface tension lowering [128, 129, 143, 145].

2.3.3.3 Inhibition Reversal

The methodology developed above allows study of inhibition reversal, i.e. the restoration of the normal surface activity of an inhibited lung surfactant film. This is illustrated in
While a mixture of BLES and protasan has been used to form the initial film so far, in this experiment a film was originally formed using serum only as the basic preparation. The serum was allowed to adsorb first to form a film at the interface and the dynamic surface tension was measured as shown in figure 2.23(a). Then the serum bulk was replaced by a preparation of BLES and protasan and the dynamic surface tension was measured again as shown in figure 2.23(b). The first few cycles are close to those before bulk replacement. Starting from the sixth cycle the minimum surface tension started to decrease from 38.0 ±0.2 mJ/m² to 5.7±0.6 mJ/m². Beyond the twelfth cycle there was no further significant change in the minimum surface tension. The changes from cycles 6 to 12 indicate that the preparation of BLES and protasan was able to replace serum components at the interface, effectively reversing the serum inhibition. A low concentration of serum (0.01 ml/ml) was used in this experiment just to illustrate the capacity of the new methodology proposed here. More detailed investigations can be readily performed.

In a recent study, spreading experiments using a Langmuir trough showed that serum (with concentrations as low as 0.0017 ml/ml) alone adsorbs to the air-water interface. When a clinical lung surfactant (Curosurf) was spread on top of the adsorbed serum film, only minimal decreases in surface tension were observed. This is similar to what is seen in figure 2.23 where the equilibrium surface tension did not change before and after the bulk replacement. However, in that previous study, only adsorption time could be investigated, not the dynamic cycling.

In conclusion, a novel methodology for studying adsorbed or spread surfactant films before and after replacing one bulk subphase with another possibly also containing surface active material was presented. This technique allowed lung surfactant films to be formed by adsorption from a surfactant subphase and to be studied on a new subphase containing inhibitor molecules rather than surfactant. Such studies were performed at physiologically relevant conditions, mimicking the leakage of plasma and blood proteins.
into the alveolar spaces. The main advantage of the new method, in addition to complete leakage elimination and controlled physiologically relevant conditions inherent in ADSA–CSD, is the ability to exchange the subphase after forming adsorbed or spread films.
Chapter 3

Dynamic Surface Tension Model*

3.1 Introduction

A key property of lung surfactants is their dynamic surface tension response to film compression and expansion. To assess the performance of surfactant preparations subject to dynamic compression/expansion, typically only the minimum surface tension at the end of compression is reported. While the minimum surface tension is useful for a preliminary assessment, it has to be realized that it depends on the method of compression [46]. Increasing the extent of compression or the speed of compression may significantly change the value of the minimum surface tension achieved [25]. In fact, this makes the comparison of different literature values difficult in the case of different compression protocols. Therefore, there is a need to concentrate on the properties of the film itself such as the elasticity and the relaxation as proposed in the literature [25, 147] rather than simply reporting the minimum surface tension achieved. In this chapter, a new approach to evaluate the quality of lung surfactant preparations is developed – beyond the $\gamma_{\min}$ value as the only quantitative characteristic – based on determining the film properties for any compression protocol.

*A portion of this chapter was previously published in Ref. [146], reproduced with permission.
In ADSA–CSD experiments, a large amount of data is collected including the surface tension as a function of time as well as the change of the drop surface area and volume. Such results can provide valuable information about various properties of the film. ADSA–CSD is used to perform dynamic cycling by successive compression/expansion of the drop via programmed cycling of the drop volume. This is normally done through 20 cycles with a periodicity of 3 seconds per cycle and a compression ratio (percentage of surface area reduction) of 20% to simulate normal human breathing.

A typical experimental result for 2.0 mg/ml BLES at 100% relative humidity and 37°C is shown in figure 3.1. Dynamic cycling starts after an equilibrium surface tension value due to adsorption is reached. The response of surface tension for a trapezoidal change of drop surface area is shown. Parenthetically, the trapezoidal perturbation of the drop volume is a consequence of the backlash of the stepping motor controlling the syringe. The fluid mechanics rounds off the corner of the trapezoidal perturbation as shown in the figure. In region A, the drop volume is decreased causing a decrease in drop surface area and a corresponding decrease in surface tension due to compression. In region B, the drop volume is kept constant while the motor is reversing its direction; therefore, the surface area is almost constant. The surface tension in this region starts to increase towards the equilibrium value. In region C, the drop volume is increased causing an increase in drop surface area and a corresponding increase in surface tension due to expansion. In region D, the drop volume is kept constant while the motor is reversing its direction to start a new cycle; therefore, the surface area is almost constant. The surface tension in this region starts to decrease towards the equilibrium value again.

The response of surface tension for every part of the trapezoidal area change is useful to understand different film properties. For regions A and C in figure 3.1, the response of surface tension to a continuous decrease (A) or increase (C) in surface area gives an indication of the dilatational elasticity of the surfactant film. For regions B and D in figure 3.1, the response of surface tension to a constant surface area gives an indication
Figure 3.1: The change of surface tension and surface area with time of one cycle for 2.0 mg/ml BLES at wet conditions, 37°C, and 20% compression ratio with a periodicity of 3 seconds per cycle.
of the interfacial adsorption/desorption of the surfactant film. If the surface tension is above the equilibrium value, adsorption is dominant (region D) and promotes a decrease in the surface tension towards the equilibrium value. However, if the surface tension is below the equilibrium value, desorption is dominant (region B) and promotes an increase in the surface tension towards the equilibrium value.

It is important to note that near the end of region A, there are two simultaneous effects: elasticity (due to the continuous decrease in surface area) and desorption (because the surface tension is below the equilibrium value). Similarly, near the end of region C, there are two simultaneous effects: elasticity (due to the continuous increase in surface area) and adsorption (because the surface tension is above the equilibrium value).

It is necessary to quantify the dynamic surface tension responses in regions A, B, C, and D through adequate models that can reflect elasticity of the lung surfactant film as well as adsorption and desorption at the interface. Such phenomena have been studied, separately, by different groups. The most relevant studies are reviewed here. However, for the case of pulmonary surfactant films, it is expected that more than one of these phenomena are acting at any given time. The combined effect can be evaluated by simple addition of individual effects. This idea is introduced and evaluated in this chapter.

The simplest possible model is considered here to explain the changes in the surface tension during the dynamic cycling based on three main well known processes: adsorption/spreading, desorption/relaxation, and elasticity/compressibility. The procedure developed in this chapter is to evaluate the quality of lung surfactant preparations – beyond the $\gamma_{\text{min}}$ value as the only quantitative characteristic – based on calculating the film properties independent of the compression protocol used. The results show that the proposed models can explain the performance of different lung surfactant preparations based on the chosen effective dynamic parameter.

The structure of this Chapter is as follows: Previous dynamic models are reviewed and summarized in Section 3.2. A proposed model called Compression–Relaxation Model
(CRM) is introduced and developed in detail in Section 3.3. Sample experiments are used with the previous models and the new developed CRM in Section 3.4 for comparison purposes. The computational details of CRM and how to use the model with experimental data are explained in the same section. Section 3.5 shows a detailed study of the dynamic surface tension (using CRM) of a lung surfactant preparation, BLES, at concentrations, compression ratios and compression rates relevant to current exogenous surfactant therapies. A detailed sensitivity study of CRM is also presented in the same section.

3.2 Previous Models

There are two approaches to describe the dynamics of adsorption at liquid/air interfaces. The diffusion controlled model assumes that the diffusional transport of interfacially active molecules through the bulk is the rate limiting process and that the adsorption is instantaneous. In the kinetic model, the adsorption and desorption steps are the rate limiting processes.

3.2.1 Diffusion Model

One of the available diffusion models assumes that the surface tension relaxation is dominated by a translational-diffusion mechanism [136]. According to this mechanism, the surfactants diffuse through the subphase to reach the air/water interface, so that the magnitude of surface tension reduction ($\Delta \gamma$) is:

\[ \Delta \gamma_1(t) = \frac{\Omega \epsilon_o}{2 \omega_o} \exp \left( 2 \omega_o t \right) \text{erfc} \left( \sqrt{2 \omega_o t} \right) + \frac{\Omega \epsilon_o \sqrt{t}}{\sqrt{2 \pi \omega_o}} - \frac{\Omega \epsilon_o}{2 \omega_o} \quad 0 < t \leq t_1 \quad (3.1a) \]

\[ \Delta \gamma_2(t) = \Delta \gamma_1(t) - \Delta \gamma_1(t - t_1) \quad t_1 < t \leq t_2 \quad (3.1b) \]

\[ \Delta \gamma_3(t) = \Delta \gamma_2(t) - \Delta \gamma_2(t - t_2) \quad t_2 < t \leq t_3 \quad (3.1c) \]

\[ \Delta \gamma_4(t) = \Delta \gamma_3(t) - \Delta \gamma_3(t - t_3) \quad t > t_3 \quad (3.1d) \]
where \( \Omega \) is the area constant, \( \epsilon_o \) is the elasticity constant, and \( \omega_o \) is the diffusion constant:

\[
\Omega = \frac{d \ln A}{dt} = \frac{1}{t_1} \ln \left(1 - \frac{\Delta A}{A_o}\right), \quad \epsilon_o = -\frac{d\gamma}{d\ln \Gamma}, \quad \omega_o = \frac{D}{2} \left(\frac{dc}{d\Gamma}\right)^2
\]  

(3.2)

where \( \Gamma \) and \( c \) are the surface and bulk concentrations, and \( D \) is the diffusion coefficient. This surface tension response is based on a trapezoidal change in surface area where \( t_1 \), \( t_2 \) and \( t_3 \) are the limits of different time domains associated with this change as shown in regions A, B, C, and D in figure 3.1.

The model parameters \( \epsilon_o \) and \( \omega_o \) (defined above) are surface thermodynamic properties. Their values, obtained from fitting different surfactants, depend on the concentration \( c \) and on the surface activity \( d\gamma/dc \). This model was compared to experiments using a variety of soluble surfactants: n-dodecyl-dimethyl-phosphine oxide (DC_{12}PO), sodium bis(2-ethylhexyl)-sulfo-succinate (DESS), and n-octadecyl-trimethyl-ammonium bromide (STAB). Good agreement was obtained between experimental data and the theoretical curves for the selected soluble surfactants [136]. Other models were also developed for soluble surfactants [148, 149]. However, the diffusion model may not be the most appropriate for insoluble surfactant systems, like lung surfactants, where the exchange of matter with sub-surface material controls the dynamic of these systems [150].

### 3.2.2 Kinetic Model

As mentioned above, other models exist beside the diffusion-controlled one to describe the adsorption kinetics and exchange of matter. Most of them are based on the Langmuir hypothesis about the nature of the adsorbed layer considering that there is an equilibrium between the subsurface and the bulk of the solution. The rate of adsorption can be written as the difference between adsorption and desorption as follows [151]:

\[
\frac{d\Gamma}{dt} = k_1c \left(1 - \frac{\Gamma}{\Gamma^*}\right) - k_2 \frac{\Gamma}{\Gamma^*}
\]  

(3.3)
where $\Gamma$ is the surface concentration, $\Gamma^*$ is the maximum equilibrium surface concentration, $c$ is the bulk concentration, $k_1$ is the adsorption coefficient, and $k_2$ is the desorption coefficient.

A model for an adsorption layer relaxation was derived [151] by considering the time-dependent square pulse area $A(t)$. In this model, the interfacial tension response can be calculated as:

$$\Delta \gamma_1(t) = \epsilon_o \frac{\Delta A}{A_o} \exp(-Kt) \quad 0 < t \leq t_1 \quad (3.4a)$$

$$\Delta \gamma_2(t) = \Delta \gamma_1(t) - \Delta \gamma_1(t - t_1) \quad t > t_1 \quad (3.4b)$$

where $t_1$ is time range of the square pulse cycle while the area is compressed, $\Delta A/A_o$ is the relative area change, $\epsilon_o$ is the elasticity coefficient and $K$ is the sorption coefficient (Langmuir Isotherm).

$$\epsilon_o = -\frac{d\gamma}{d\ln \Gamma} = RT \frac{\Gamma^*/\Gamma^*}{1 - \Gamma/\Gamma^*}, \quad K = \frac{k_1c}{\Gamma^*} + \frac{k_2}{\Gamma^*} \quad (3.5)$$

Equation 3.4 can be used to interpret experimental data by the use of a fitting procedure. It is important to note that equation 3.4 suggests that adsorption and relaxation are first order processes. The resulting values for $\epsilon_o$ and $K$ are obtained independently of any knowledge of the parameters of the equilibrium adsorption isotherm. On the other hand, both parameters contain specific constants of the adsorption isotherm and are therefore of interest also from this point of view.

The results of relaxation experiments of human albumin (HA) adsorbed at the aqueous solution/air interfaces [152] were discussed on the basis of the diffusion model as shown in equation 3.1. The resulting diffusion coefficients were found to be two to three orders of magnitude higher than the physically expected value. It was concluded that the adsorption layer of human albumin at the aqueous solution/air interface shows a dy-
namic behaviour similar to that of surfactants, while relaxations after area disturbances are reversible and controlled by a mechanism other than diffusion.

The same experimental data were interpreted using both models \[151\]: the diffusion model (equation 3.1) and the kinetic model (equation 3.4). It was shown that the kinetic model yields values of the parameters which are rather consistent (considering different cycles of the same run) while those obtained from the diffusion model are more scattered.

### 3.2.3 Adsorption-Limited Model

In another model, transport of surfactant to the interface is assumed to be adsorption-limited as opposed to diffusion-limited \[153\]. Several assumptions were adopted; one is that there exists a maximum surfactant concentration to which the interface can be dynamically packed, and when interfacial area is reduced from this point, surfactant is squeezed out from the surface. Another assumption is that transport of surfactant to the interface is adsorption rather than diffusion limited. When the interfacial concentration of surfactant is less than its maximum equilibrium value, adsorption and desorption are controlled by Langmuir kinetics, while for interfacial surfactant concentrations between the maximum equilibrium value and the maximum packing concentration, the surfactant is frozen in the surface and can neither adsorb nor desorb. The kinetics of adsorption and desorption were defined in this model by three interfacial surface concentration (\(\Gamma\)) regimes:

\[
\begin{align*}
\frac{d(A\Gamma)}{dt} &= A\left[k_1 C(\Gamma^* - \Gamma) - k_2 \Gamma\right] & \Gamma < \Gamma^* & \text{(3.6a)} \\
\frac{d(A\Gamma)}{dt} &= 0 & \Gamma^* < \Gamma < \Gamma_{\text{max}} & \text{(3.6b)} \\
\frac{d(A\Gamma)}{dt} &= -\Gamma_{\text{max}} \frac{dA}{dt} & \Gamma = \Gamma_{\text{max}} & \text{(3.6c)}
\end{align*}
\]

where \(k_1\) is the adsorption coefficient, \(k_2\) is the desorption coefficient, \(C\) is the bulk concentration, \(\Gamma^*\) is the maximum equilibrium surface concentration, and \(\Gamma_{\text{max}}\) is the
maximum dynamic surface concentration (collapse).

The relationship between the normalized interfacial concentration, $\Gamma/\Gamma^*$, and the surface tension, $\gamma$, is the compression isotherm. The compression isotherm is modeled as two straight lines that meet at $\Gamma = \Gamma^*$, the slopes for both regions are $m_1$ and $m_2$. Therefore, surface tension can be calculated as follows:

\[
\gamma = \gamma_o - m_1 \frac{\Gamma}{\Gamma^*} \quad \text{for} \quad \frac{\Gamma}{\Gamma^*} < 1.0 \quad (3.7a)
\]

\[
\gamma = \gamma^* - m_2 \left( \frac{\Gamma}{\Gamma^*} - 1 \right) \quad \text{for} \quad \frac{\Gamma_{\text{max}}}{\Gamma^*} \geq 1.0 \quad (3.7b)
\]

where $\gamma_o$ is the water surface tension, $\gamma^*$ is the equilibrium surface tension, $m_1$ is the isotherm slope for $\gamma > \gamma^*$, and $m_2$ is the isotherm slope for $\gamma_{\text{min}} < \gamma < \gamma^*$.

This model [153] was used to characterize the dynamic behaviour of lung surfactant fractions [154], containing: (1) phospholipids (PL) only, (2) PL and hydrophobic apoproteins (HA), (3) PL and HA and surfactant protein A (SP-A), and (4) PL and HA and SP-A and neutral lipids (NL). The results were compared to those of native surfactant and dipalmitoyl-phosphatidyl-choline (DPPC). The effect of individual components on the surface activity (minimum surface tension, adsorption, film compressibility) of the mixture was evaluated by comparing the experimental results to the model predictions. It was noted that the model produces predictions that are qualitatively but not quantitatively close to the experimental results for most of the cases.

The adsorption-limited model [153] was later extended to characterize the initial transient behaviour (that occurs upon initiation of pulsation, before steady-state was reached) by including the effects of diffusion in the bulk phase and comparing the results to both transient and steady-state oscillatory data [155]. It was concluded that at steady-state cycling conditions, diffusion-limited transport is most relevant. While there are a number of situations in which the extended model gives significantly improved predictions, steady-state oscillatory data at high bulk concentration are still adequately modeled by
an adsorption-limited model, which is computationally more efficient. When compared to previous experimental data [154], both models yield results that are very close to each other but are far from the experimental results.

### 3.3 Development of Compression–Relaxation Model

As inferred from the dynamic experiments, there are four main factors that affect the response of the dynamic surface tension: adsorption or spreading, desorption or relaxation, elasticity during compression and elasticity during expansion. In the proposed model, the adsorption and relaxation rates depend on how far the surface tension is from the equilibrium value while the elasticity for both compression and expansion depends on changes in the surface area of the interface. The main feature is to allow the surface tension to change due to simultaneous effects of one or more of these parameters.

#### 3.3.1 Adsorption Process (Spreading)

Based on the Langmuir hypothesis, the rate of adsorption can be written as the difference between adsorption and desorption as shown in equation 3.6a. As mentioned before, $\Gamma^\ast$ is the maximum equilibrium interfacial concentration, i.e. the limiting value of $\Gamma_{eq}$ reached as $c$ (concentration) is increased. Also, $\Gamma_{eq}$ can be calculated as [153]

$$\frac{\Gamma_{eq}}{\Gamma^\ast} = \frac{k_1 c}{k_1 c + k_2}$$

(3.8)

Note that equation 3.6a predicts adsorption for surface concentration less than the equilibrium concentration, $\Gamma_{eq}$, and desorption for concentrations greater than this. Therefore, for surface concentration less than $\Gamma_{eq}$ (surface tension greater than $\gamma_{eq}$) and for a given concentration and constant surface area, that equation can be simplified to

$$\frac{d\Gamma}{dt} = k(\Gamma_{eq} - \Gamma)$$

(3.9)
where $k$ is a coefficient for the dynamic adsorption and desorption. This indicates that both rates of adsorption/spreading and desorption/relaxation are proportional to the difference between the surface coverage at any time and the equilibrium surface coverage. However, since the mechanisms of adsorption and desorption for pulmonary surfactant compounds are different, we use here two coefficients, one for adsorption and another for desorption.

Assuming a linear relationship between the surface concentration and the surface pressure in the region above the equilibrium surface tension (equivalent to the assumptions behind equation 3.7 in the adsorption-limited model [153]), equation 3.9 can be further simplified to

$$\frac{d\gamma}{dt} = k_a(\gamma_{eq} - \gamma) \quad \text{for } \gamma \geq \gamma_{eq} \quad (3.10)$$

where $k_a$ is the adsorption coefficient.

It is important to note that the above equations were derived for a single component system. It is assumed here that it is also valid for multi-component systems, where the coefficient now has to be understood as an effective constant for the system, not any specific material. The comparison between experimental results and the developed model will show whether this assumption is reasonable.

### 3.3.2 Desorption Process (Relaxation)

As mentioned earlier, equation 3.6a predicts adsorption for surface concentration less than the equilibrium concentration, $\Gamma_{eq}$, and desorption for concentrations greater than this. Therefore, for surface concentration greater than $\Gamma_{eq}$ (surface tension less than $\gamma_{eq}$) and constant surface area, a simplified desorption relation similar to that of equation 3.10 can be used,

$$\frac{d\gamma}{dt} = k_r(\gamma_{eq} - \gamma) \quad \text{for } \gamma \leq \gamma_{eq} \quad (3.11)$$

where $k_r$ is the desorption or relaxation coefficient.
Non-equilibrium surface pressures occurring during dynamic compression are believed to indicate the formation of metastable surface states within phospholipid films [1]. When the compression is stopped (the surface area is kept constant), the dynamic surface pressure relaxes towards the equilibrium values. Relaxation has also been studied [156–158] in terms of a nucleation mechanism [159–161] through which a similar expression to equation 3.11 can be derived. After compression beyond collapse, the dynamic surface pressure becomes more stable and remains in the vicinity of the dynamic collapse value over an extended time scale [162].

### 3.3.3 Elasticity

The dilational interfacial elasticity is defined by [136, 151],

\[ \epsilon_o = -\frac{d\gamma}{d\ln A} \] (3.12)

which is equivalent to

\[ \frac{d\gamma}{dt} = -\epsilon_o \left( \frac{1}{A} \frac{dA}{dt} \right) \] (3.13)

If we consider different elasticities for compression and expansion, the above equation becomes,

\[ \frac{d\gamma}{dt} = \begin{cases} \epsilon_c \left( \frac{1}{A} \frac{dA}{dt} \right) & \text{for } \frac{dA}{dt} \leq 0 \\ \epsilon_e \left( \frac{1}{A} \frac{dA}{dt} \right) & \text{for } \frac{dA}{dt} \geq 0 \end{cases} \] (3.14)

### 3.3.4 Collapse

It is well known that many surfactant layers at the interface can be compressed to pressures considerably higher than their equilibrium spreading pressure [1, 92, 93]. As discussed in Chapter 2 if compression continues beyond an ultimate collapse pressure, the surfactant layers collapse ejecting surfactant molecules into one or more surface or subsur-
face collapse structures or phases \[1, 93\]. Therefore, once the ultimate collapse pressure, i.e. the minimum surface tension, is reached, the surface tension can not be further decreased by more compression. This can be formulated as follows

\[
\frac{d\gamma}{dt} = 0 \quad \text{for } \gamma \leq \gamma_{\text{min}} \tag{3.15}
\]

### 3.3.5 The Integrated Model

Combining equations 3.10, 3.11, 3.14, and 3.15 and allowing the surface tension to change due to simultaneous effects of relaxation/adsorption and elasticity, we obtain:

\[
\frac{d\gamma}{dt} = \begin{cases} 
\frac{d\gamma_1}{dt} + \frac{d\gamma_2}{dt} & \text{if } \gamma \geq \gamma_{\text{min}} \\
0 & \text{if } \gamma \leq \gamma_{\text{min}}
\end{cases}
\tag{3.16a}
\]

where

\[
\frac{d\gamma_1}{dt} = \begin{cases} 
k_a (\gamma_{eq} - \gamma) & \text{if } \gamma \geq \gamma_{eq} \\
k_r (\gamma_{eq} - \gamma) & \text{if } \gamma \leq \gamma_{eq}
\end{cases}
\tag{3.16b}
\]

\[
\frac{d\gamma_2}{dt} = \begin{cases} 
\epsilon_c \left(\frac{1}{A} \frac{dA}{dt}\right) & \text{if } \frac{dA}{dt} \leq 0 \\
\epsilon_e \left(\frac{1}{A} \frac{dA}{dt}\right) & \text{if } \frac{dA}{dt} \geq 0
\end{cases}
\tag{3.16c}
\]

According to this model better lung surfactant preparations can be identified as having faster adsorption rate (higher \(k_a\)), slower relaxation rate (lower \(k_r\)), and higher elasticity of compression and expansion (higher \(\epsilon_c\) and \(\epsilon_e\)). Figure 3.2 shows a comparison between two hypothetical dynamic surface tension responses, where one has desired dynamic properties and the other does not. The figure shows schematics based on an assumed, i.e. exact, trapezoidal change in surface area. However, it is important to note that the proposed model does not assume any specific perturbation waveform and can be applied
to various perturbations including trapezoidal and sinusoidal.

These dynamic parameters are actually very similar to those used in the previous models. The elasticities of compression and expansion, $\epsilon_c$ and $\epsilon_e$, are defined in the same fashion as the elasticity constant, $\epsilon_o$, in both the diffusion model \[136\] and the kinetic model \[151\]. The adsorption coefficient, $k_a$, is similar to that used in the adsorption-limited model \[153\]. Thus, the equations involving these parameters are not novel, but it is the simultaneous solution for these parameters that makes this model unique. The model introduces the possibility of simultaneous compression and relaxation effects. It is important to note that the calculations of these parameters in the current model are based directly on the surface tension rather than the surface concentration. This allows the direct use of ADSA output (changes in surface tension and surface area with time) in the fitting procedure to estimate the film properties.

3.4 CRM Applied to Sample Experiments

3.4.1 Experimental Details

Details about the experimental setup and procedure were given in Chapter \[2\]. During the experiment, the setup is enclosed in an environmental control chamber that facilitates the control of gas composition and temperature. The humidity inside the chamber was kept constant (wet at 100% relative humidity or dry < 20% relative humidity) at 37°C. The dynamic cycling experiments were carried out by periodically injecting/withdrawing liquid from the drop. After reaching the equilibrium surface tension value, dynamic cycling is started. The dynamic cycling consists of four sub-stages: compression, relaxation, expansion and re-adsorption, as indicated in the discussion of figure \[3.1\]. Throughout Section \[3.4\], the compression conditions were: compression periodicity of 3 s/cycle, and compression ratio (fraction of surface area reduction) of 20% to simulate normal breathing conditions \[11, 163\].
Figure 3.2: The response of surface tension to a trapezoidal change in surface area using the new compression-relaxation model for a good (solid line) and a bad (dashed line) system: (a) the trapezoidal change of surface area with time; (b) the response of surface tension with time for both systems; (c) the change of surface tension with the relative surface area for both systems. The good system (solid line) is generated using parameters: $\epsilon_c = 150 \text{ mJ/m}^2$, $\epsilon_e = 150 \text{ mJ/m}^2$, $k_r = 0.1 \text{ s}^{-1}$, $k_a = 5 \text{ s}^{-1}$. The bad system (dashed line) is generated using parameters: $\epsilon_c = 70 \text{ mJ/m}^2$, $\epsilon_e = 70 \text{ mJ/m}^2$, $k_r = 0.3 \text{ s}^{-1}$, $k_a = 0.1 \text{ s}^{-1}$. 
It was shown in recent studies [25, 46, 47] that BLES films are less stable in humid environments than in dry ones and tend to have lower surface activity compared to BLES films in dry air. To further investigate the effect of humidity, five formulations were used for model comparisons: 0.5 mg/ml BLES (humid air) [25, 91], 0.5 mg/ml BLES (dry air) [25, 91], 2.0 mg/ml BLES (humid air) [91], 2.0 mg/ml BLES (dry air) [91], and 0.5 mg/ml BLES mixed with 2.5 mg/ml Albumin (humid air) [47]. The last formulation is chosen to illustrate the effect of a known inhibitor on the surface activity and film properties of BLES.

3.4.2 Computational Details

The proposed compression/relaxation model, as summarized in equation 3.16, is able to predict the surface tension response of a surfactant preparation for a given change in surface area with time, assuming an a priori knowledge of the above mentioned four dynamic parameters: adsorption coefficient ($k_a$), desorption coefficient ($k_r$), elasticity of compression ($\epsilon_c$), and elasticity of expansion ($\epsilon_e$). The numerical procedure for determining these four parameters requires three steps: First, the differential equation 3.16 must be solved using numerical integration to generate a surface tension response. Second, initial guesses for the four parameters are needed; the experimental results are analyzed to calculate approximate values (initial guesses) for these parameters. Third, an optimization procedure is required to compare the calculated surface tension values with the experimentally measured values, and modifying the parameter values until a matched surface tension response is obtained. These three steps are explained in detail below.

3.4.2.1 Numerical Integration

Equation 3.16 is considered as a set of ordinary differential equations and can be solved using an initial value problem solver for given values of the four dynamic parameters. A variable order Adams-Bashforth-Moulton PECE solver [164] is implemented for the
numerical integration. This method is considered more efficient than the Runge-Kutta solver at stringent tolerances and when the ordinary differential equations set is particularly expensive in computation time. This solver is a multi-step solver; it normally needs the solutions at four preceding time points to compute the solution at a specific point in time. The first point is usually the first surface tension value measured experimentally. The following three points are usually calculated using the Runge-Kutta solver and then the Adams-Bashforth-Moulton solver is used afterwards.

3.4.2.2 Initial Guess

As mentioned earlier, an initial guess for the dynamic parameters is required for the curve fitting module. Simple methods previously used to calculate dilatational elasticity, adsorption, and relaxation rates in the literature [25, 47, 91] are used to estimate initial values.

For the elasticity of compression and expansion, the change of surface tension with the relative surface area during the compression/expansion part of every cycle is fitted to a fourth order polynomial and the slope is evaluated at the midpoint [91]. The calculated slope and the value of the relative surface area are used to calculate the dilatational elasticity according to equation 3.12. This is similar to what was used in Chapter 2.

For the adsorption and desorption coefficients, the surface tension increase/decrease with time (region B and D in figure 3.1) is fitted to a fourth order polynomial and the slope is evaluated at the midpoint [91]. The calculated slope and the difference between the surface tension value and the equilibrium surface tension value are used to calculate the adsorption and desorption coefficients according to equations 3.10 and 3.11.

3.4.2.3 Curve Fitting (Optimization Procedure)

Once the initial guesses for the dynamic parameters are calculated, the numerical integration is performed and the calculated surface tension response is compared to the
experimental values using a curve fitting module (optimization procedure). Multidimensional unconstrained nonlinear minimization (Nelder-Mead) is used to find the minimum of a fitting residual function, \( \mathcal{R} \) (see equation 3.17 below), using the simplex derivative-free search method \[165\]. This is a direct search method that does not use numerical or analytic gradients. At the end of this module, the four dynamic parameters are obtained from the experimental result of a specific cycle. The same procedure is applied for different cycles (typically 20 cycles) and the values reported below are the average value and the standard deviation for a specific preparation.

### 3.4.3 Results

The proposed compression-relaxation model for dynamic characterization is implemented using the above mentioned numerical details. Four preparations (high and low concentration of BLES with humid and dry conditions) were selected to illustrate the significance of different parameters. Figure 3.3 shows the change of surface tension with the relative surface area of one cycle for these preparations. The compression/expansion cycles of surface layers are commonly used for qualitative and quantitative data analysis as proposed in the literature \[166\]. The model predictions fit most of the experimental points well. Figure 3.4 shows the change of surface tension and surface area with time for the same four preparations. In the same figure, the compression-relaxation model is compared to other models, i.e. the adsorption-limited model and the diffusion model. The kinetic model was considered but can not be compared because it was designed to work only with square pulse changes in surface area, which is not the case for the experiments considered here. The results in figure 3.4 show that the proposed compression-relaxation model has better quantitative agreement with experimental results than other models.

A special case is shown in figure 3.5, where the film has collapsed at a high surface tension due to the presence of albumin in BLES. The proposed compression-relaxation model shows again a better fit with the experimental points compared to other models.
Figure 3.3: The change of surface tension with the relative surface area of one cycle for four different preparations of diluted and concentrated BLES in humid (H) and dry (D) conditions. The compression-relaxation model (CRM) predictions is compared to the experimental points.
Figure 3.4: The change of surface tension with time of one cycle for four different preparations of diluted and concentrated BLES in humid (H) and dry (D) conditions. The compression-relaxation model (CRM) predictions is compared to the adsorption-limited model (ALM), the diffusion model (DM), and the experimental points.
A summary of the four dynamic parameters calculated from the compression-relaxation model for the different five preparations is shown in table 3.1. The third column of the table shows the values of elasticity of compression calculated from an elementary procedure used previously in the literature [91] and in Chapter 2. The values in this table will be discussed in detail in the next section.

Figure 3.6 compares the values of the fitting residual function from different models as an indication of the goodness of fit. The fitting residual function is defined as:

$$\mathcal{R} = \sqrt{\sum |\gamma_e - \gamma_m|^2}$$  

(3.17)

where $\gamma_e$ is the experimentally measured surface tension value and $\gamma_m$ is the predicted surface tension value from the respective model. Five cases (the four cases of diluted and concentrated BLES in humid and dry conditions shown in figure 3.3 and the case of adding albumin shown in figure 3.5) were considered here. It is apparent that the compression-relaxation model gives the best fit for all five cases. The adsorption-limited model performed very well in the second case (film collapse at low surface tension with minimum relaxation) but performed poorly in the third case (relaxation process with no collapse).

Figure 3.7 shows a comparison between the calculated values of elasticity between different models. In the proposed compression-relaxation model, two different values were considered (one for compression and another for expansion). It is noted that the difference between calculated elasticity values are not very big except for the diffusion model in two of the five cases.
Figure 3.5: A special case of collapse at high surface tension of diluted BLES mixed with albumin in humid (H) condition: (a) the change of surface tension with the relative surface area of one cycle; (b) the change of surface tension and surface area with time of one cycle. CRM: compression-relaxation model, ALM: adsorption-limited model, DM: diffusion model.
Table 3.1: Summary of the dynamic parameters (mean and standard deviation) calculated from the compression-relaxation model for different preparations at 20% compression and 3 seconds per cycle.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Humidity Condition</th>
<th>Ref. [91]</th>
<th>CRM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Elasticity of Compression $\epsilon_c$ (mJ/m$^2$)</td>
<td>Elasticity of Expansion $\epsilon_e$ (mJ/m$^2$)</td>
</tr>
<tr>
<td>BLES 0.5 mg/ml</td>
<td>Humid</td>
<td>99.8±2.9</td>
<td>125.1± 3.8</td>
</tr>
<tr>
<td>BLES 0.5 mg/ml</td>
<td>Dry</td>
<td>105.9±3.8</td>
<td>112.7± 1.4</td>
</tr>
<tr>
<td>BLES 2.0 mg/ml</td>
<td>Humid</td>
<td>49.1±8.7</td>
<td>126.3± 2.8</td>
</tr>
<tr>
<td>BLES 2.0 mg/ml</td>
<td>Dry</td>
<td>108.9±5.3</td>
<td>123.1± 2.1</td>
</tr>
<tr>
<td>BLES 0.5 mg/ml &amp;</td>
<td>Humid</td>
<td>10.3±2.6</td>
<td>72.4±10.6</td>
</tr>
<tr>
<td>Albumin 2.5 mg/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Experimental results used here were taken from the literature [25, 47, 91].
Figure 3.6: Comparison between the goodness of fit (mean and standard deviation) of different models. CRM: compression-relaxation model, ALM: adsorption-limited model, DM: diffusion model.
Figure 3.7: Comparison between the predicted value of elasticity (mean and standard deviation) using different models. CRM: compression-relaxation model, ALM: adsorption-limited model, DM: diffusion model.
3.4.4 Discussion

3.4.4.1 Comparison of Models

The compression-relaxation model presented here shows better fitting than other models for the five experimental cases shown here. The model can quantify the dynamic parameters that affect the response of the dynamic surface tension: adsorption coefficient, desorption or relaxation coefficient, and dilatational elasticity of compression and expansion. The main feature in this model is that more than one process is occurring at any given time (compared to different regions of specific mechanism for each). This is evident, for example, in the experimental result of humid concentrated BLES, figure 3.3(c), where the surface tension starts to increase near the end of the compression stage indicating a relaxation process occurring during the change in area not only when the area is constant. This suggests a combined and simultaneous effect of relaxation and elasticity. A similar effect is observed near the end of the expansion stage of the same experiment, where the surface tension starts to decrease, suggesting that surfactant adsorption is already occurring during expansion.

The diffusion model did not show good agreement with most of the experimental results. The main assumption in that model is that the diffusion of surfactant molecules through the subphase to reach the interface is the dominant physical phenomenon. In this model, the diffusion coefficient is the same for diffusion to or from the interface. This would apply to soluble surfactants like DC\textsubscript{12}PO, DESS and STAB \cite{136}. However, pulmonary surfactants are insoluble surfactants suspended as vesicles or other aggregates and spread at the interface forming possibly multilayers \cite{137,139,167}.

The adsorption-limited model did show a reasonable agreement with experimental results for some of the cases. However, for humid diluted and humid concentrated BLES, the adsorption-limited model was not able to capture the desorption/relaxation phenomenon. According to this model, the surfactant can only collapse, i.e. be squeezed
out, but can neither adsorb nor desorb below the minimum equilibrium surface tension, \( \gamma^* \). The better agreement with experimental results for dry diluted and concentrated BLES is likely due to the fact that relaxation is not pronounced in these cases.

### 3.4.4.2 Calculated Dynamic Parameters

Besides the minimum surface tension value, the calculated dynamic properties can be considered a measure of the quality of the lung surfactant preparation. For example, with respect to the calculated elasticity value for all five preparations shown in figure 3.7 and table 3.1 results suggest that there is not much variation in the elasticity values calculated for humid and dry, diluted and concentrated BLES. However, when albumin is added to BLES, the elasticity value is markedly decreased from approximately 125 to 75 mJ/m\(^2\), indicating surfactant inactivation or inhibition. This conclusion agrees with findings in the literature \[34, 76, 116, 117, 122, 168–170\] that albumin poisons surface activity of lung surfactant preparations.

For most of the preparations studied, the values obtained for the elasticities of compression and expansion are quite close except for the case of diluted BLES at high humidity. The difference between the values of elasticity calculated during compression and expansion may suggest that the film composition during compression is not the same as during expansion, which is consistent with the hypothesis that some less surface active material is squeezed out of the film \[1, 171, 172\]. Such changes in the film composition may happen during the course of compression or just at the end of the compression. These possibilities should be further investigated. A hydration-film fluidization mechanism has been proposed to explain the effect of humidity on the film elasticity \[25\]. However, this effect is minimal when the compression periodicity is short enough for the hydration effects not to take place\((\sim3\text{ s/cycle})\) and the elasticity remains almost constant \[25\].

The desorption (relaxation) coefficient calculated here is also an important quality
measure for lung surfactant preparations. Comparing the calculated values for diluted and concentrated BLES in table 3.1, it is clear that the relaxation coefficient increases with the increase of concentration for both humid and dry conditions. However, there is a distinctive effect of humidity on the calculated value of the relaxation coefficient. For both diluted and concentrated BLES, the dry condition causes a more stable preparation; the relaxation coefficient is two orders of magnitude smaller than in the case of humid conditions. This indicates that little or no significant relaxation is experienced by BLES films in dry air as suggested in the literature on the basis of the hydration-film fluidization mechanism [25]. Comparing the diluted humid BLES to the same preparation mixed with albumin, the increase of the relaxation coefficient is another indication of lung surfactant inhibition.

In summary, the dynamic compression-relaxation model is used to describe the mechanical properties of insoluble pulmonary surfactant films by investigating the response of surface tension to changes in surface area. This model introduces the possibility of simultaneous compression and relaxation effects. The model is able to fit different experimental results of diluted and concentrated BLES preparation evaluated in humid and dry air, as well as BLES and albumin mixtures. The calculated dynamic parameters confirm the known effect of albumin on changing the elasticity and desorption of BLES preparations. The effect of humidity on the desorption process is illustrated by the huge increase in the relaxation coefficient in humid conditions, indicating that the dry condition causes a more stable surfactant films. Since the humid conditions are physiologically relevant to lung surfactant studies, the effect of humidity introduces some doubts and questions regarding the optimistic results of lung surfactant films in dry conditions in the literature.
3.5 Effect of Concentration, Compression Ratio and Compression Rate

In this section, ADSA–CSD is used to measure the dynamic surface tension of a lung surfactant preparation, BLES, at concentrations, compression ratios and compression rates relevant to current exogenous surfactant therapies. The results are analyzed using CRM to evaluate the effect of surfactant concentration, compression ratio and compression rate on the surface activity and dynamic properties (elasticity, adsorption and relaxation) of BLES preparations.

As indicated in Chapter 1, exogenous surfactant replacement therapy, in which either synthetic or modified natural pulmonary surfactant (extracted from bovine or porcine sources) is delivered into the patients’ lungs, has been established as a standard therapeutic intervention for patients with nRDS [173]. Surfactant therapy has shown only limited therapeutic effect on ARDS patients [174,177]. In these therapies, high concentrations of the exogenous surfactant are commonly used to reach an effective dose, i.e. the surfactant dose to recover the mechanical properties of the lungs. The dose is usually in the range of 8 mg/ml [1,178,179]. Some other formulations, e.g. bovine lipid extracted surfactant (BLES) preparations, are used in surfactant replacement therapy with larger doses of 27 mg/ml [25]. However, there is a lack of in vitro studies for these high concentrations due to limitations of methodologies used. For these studies, the maximum surfactant concentration is usually restricted to no more than 3 mg/ml. This restriction arises from optical limitations since surfactant suspensions become murky and eventually opaque at increased concentrations.

Although there is promising evidence for surfactant therapy in ARDS, the effectiveness of high doses of commercial surfactant preparations has been inconsistent in clinical trials [177,180,184]. However, surfactant concentration is only one strategy to address the problem. High frequency low tidal volume ventilation, henceforth sim-
 ply referred to as high frequency ventilation (HFV) has been shown to reduce, even if marginally, the mortality of ARDS [181, 185–189]. To simulate normal breathing, cycling frequencies of 0.1 - 0.5 Hz and a reduction in surface area to near 20% are necessary [1, 25, 36, 172, 190, 191]. However, for high frequency ventilation it is necessary to produce cycling frequencies higher than 1 Hz [192].

As indicated in Chapter 1, ADSA–CSD is capable of measuring surface tension of lung surfactants at a wide range of concentrations, compressions ratios and frequencies. In fact, in contrast to other methodologies, there is no upper limit to the surfactant concentration that can be used in ADSA–CSD. In this section, ADSA–CSD is used in conjunction with CRM to measure the effective dynamic properties of BLES at conditions relevant to conventional (high concentration) and high frequency surfactant therapies. Results show that CRM is generally sensitive to all the parameters, i.e., elasticity, adsorption and relaxation, at any concentration at physiological conditions of 20% compression and 3 seconds per cycle. The model is also very sensitive to all the parameters at any concentration at low compression ratios and high frequency cycling. These later conditions are relevant to the clinical practice of high frequency ventilation.

3.5.1 Experimental Details

Details about preparation of BLES, the experimental setup and procedures are given in Chapter 2. During the experiment, the setup is enclosed in an environmental control chamber that facilitates the control of gas composition and temperature. The humidity inside the chamber was kept constant at 100% relative humidity at 37°C. Drop images were acquired throughout the experiment at a rate of 20 images per second for cycling speeds of 3 or 9 seconds per cycle and at a rate of 30 images per second for a cycling speed of 1 second per cycle.

The experimental procedure was as follows: First, the sessile drop was quickly formed (within 0.5 s) on the pedestal. The drop was then left undisturbed to allow adsorption
of the lung surfactant film. The surface tension was tracked until the film reaches the
equilibrium surface tension (within 180 seconds for all cases). Thereafter, dynamic cycling
was performed by successive compression/expansion of the drop. This is normally done
through 20 cycles with the required periodicity (1, 3, or 9 seconds per cycle) and the
required compression ratio (percentage of surface area reduction) of 10%, 20%, or 30%.
During every experimental run, images were acquired, stored and later analyzed with
ADSA. All experiments were reproduced at least in triplicate. A typical experimental
result for 2.0 mg/ml BLES at 100% relative humidity, 20% compression, 3 seconds per
cycle and 37°C is shown in figure 3.1 showing an example of ADSA outputs as a function
of cycling time. It can be seen that cycles repeat very well. Using the change of surface
tension and area with time during dynamic cycling, dynamic parameters are calculated
using the compression-relaxation model (CRM).

3.5.2 Results

The dynamic cycling for a specific concentration is performed by successive compression/-
expansion of the drop with the prescribed periodicity and the preselected compression
ratio (percentage of surface area reduction). In this section, 36 experiments are reported:
4 concentrations (2, 8, 15, or 27 mg/ml), 3 periodicities (1, 3, or 9 seconds per cycle),
and 3 compression ratios (10%, 20%, or 30%); each experimental run contains 20 cycles
and is repeated at least 3 times. In total, 108 runs were performed.

During every experimental run, images are acquired continuously at a rate of 30
images per second (for periodicity of 1 second per cycle) or 20 images per second (for
periodicities of 3 and 9 seconds per cycle). A typical run contains approximately 600
images (20 cycles, 1 second per cycle and 30 images per second), 1200 images (20 cycles, 3
second per cycle and 20 images per second) or 3600 images (20 cycles, 9 second per cycle
and 20 images per second). Thus, results reported here rely on almost 200,000 individ-
ual surface tension measurements, involving the analysis of that number of drop images.
These images are stored and later analyzed with ADSA. For every image, ADSA calculates the surface tension, the surface area, and the drop volume as shown in figure 3.1. The reproducibility between cycles is apparent from figure 3.1.

To deal with such a huge number of data, an overall upgrade of the numerical work was performed to facilitate the efficient operation and post-analysis functions. After running all the acquired images through ADSA, an output file was created; this file needs some careful data analysis. The objectives of the data analysis are editing the ADSA output file, plotting the change of the surface tension, area and volume with time, and some preliminary analysis. This includes separating the compression and expansion parts of every cycle, plotting the change in the surface tension with the relative area for every half cycle and calculating minimum surface tension values in the cycling part. These tasks were so far performed manually consuming more than a complete day to produce the required plots and calculations for a cycling experiment. A Matlab code was developed to perform the data analysis and plotting for ADSA output files rapidly and efficiently. The code was designed to process more than one ADSA output file (batch mode for processing different experimental runs, so all input data are preselected by the user through script parameters). The outputs from this code are two plots that are saved in the same folder of the ADSA output files, and an output structure that contains: the alphabetic run indicator, compression ratio, minimum surface tension mean value, minimum surface tension standard deviation, maximum surface tension mean value and maximum surface tension standard for the specified run. The code was also extended to include the calculated dynamic parameters from CRM.

The minimum value for surface tension for every cycle can be obtained by inspection from the output of ADSA. A typical run includes 20 values for the minimum surface tension. The reported minimum surface tension value is the average value within a particular run. For a specific condition (concentration, compression ratio, and compression rate), at least three runs were performed. From every run, an average minimum surface
Figure 3.8: The minimum surface tension, $\gamma_{min}$, measured for four different concentrations of BLES (2, 8, 15, 27 mg/ml) at different compression ratios (10%, 20%, 30%) and different cycling conditions (1, 3, 9 s/cycle). The error bars indicate the standard deviation between runs repeated under the same conditions.
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tension is reported in figure 3.8.

Figure 3.8 shows the average minimum surface tension, \( \gamma_{\text{min}} \), measured for four different concentrations of BLES (2, 8, 15, 27 mg/ml) at different compression ratios (10%, 20%, 30%) and different cycling conditions (1, 3, 9 s/cycle). The standard deviation indicated by the error bars shows good reproducibility between runs repeated under the same conditions.

At higher concentration, the minimum surface tension is sensitive to changes in periodicity only at small compression ratios. At 10% compression, the minimum surface tension decreases with the increase of concentration and with the increase with cycling rate. For 20% compression and above, the minimum surface tension decreases in the range of 2 mg/ml to 8 mg/ml but stays essentially constant at higher concentrations. However, for concentrations above 8 mg/ml, there is no significant change in minimum surface tension. In clinical practice, high concentration BLES of 27 mg/ml is used \[1, 178\]. and it is believed to be diluted after delivery to lungs to a concentration close to 8 mg/ml \[179, 193–199\].

Generally, increasing the extent of compression produces lower minimum surface tension values. This agrees with previous investigations of BLES at 0.5 and 5 mg/ml and cycling speeds of 3 and 10 seconds per cycle \[25\]. At high concentrations, the speed of cycling does not have a significant effect on the minimum surface tension. The decrease of minimum surface tension with the increase of the speed of compression at low concentrations agrees with previous investigations in the literature of BLES 0.5 mg/ml and 20% compression \[25\]. This dependence of minimum surface tension on speed is probably due to the fact that the speed of compression seems to be fast enough to prevent, in part, the hydration of the surfactant film \[25\].

The surface tension measurements of BLES at these high concentrations are now possible due to the features of the ADSA–CSD methodology as mentioned before. It is also important to note that ADSA–CSD studies can be performed readily at high rates of
compression (to mimic human breathing at 3 seconds per cycle). In this study, higher rates are also reported (up to 1 second per cycle or 1 Hz). Even higher rates of compression will become achievable after hardware upgrades for both the liquid control and image acquisition systems. It is noted that flow conditions and viscosity may introduce errors in the measurement of surface tension via drop/shape methods when the cycling frequencies are 10 Hz or larger [27, 200–202]. Therefore, an upper limit of 10 Hz exists for the high frequency studies.

Using the output of ADSA for every experimental run, the change of surface tension and surface area with time are analyzed using CRM following the procedure explained in section 3.4.2. As mentioned earlier, a typical experimental run consists of 20 cycles. From every cycle, the four dynamic parameters of CRM are evaluated, i.e. elasticity of compression ($\epsilon_c$), elasticity of expansion ($\epsilon_e$), desorption/relaxation coefficient ($k_r$), and adsorption coefficient ($k_a$). An indication of the goodness of fit is also calculated for every cycle. The goodness of fit is defined below in equation 3.18, indicating the relative error between the predicted surface tension response from CRM and the experimentally measured surface tension response. Smaller values for the goodness of fit indicate a better fit.

Figures 3.9 and 3.10 show sample experimental results of the change in surface tension with time and with relative surface area during dynamic cycling. Figure 3.9 shows results of BLES concentrations 2 and 27 mg/ml at 10% compression and periodicities of 1 and 9 seconds per cycle. Figure 3.10 shows corresponding results at 30% compression. The figures illustrate the quality, or “goodness of fit” of CRM to the experimental data. In these figures, the symbols represent the measured experimental results and the continuous lines represent the best-fit CRM curves, the four parameters ($\epsilon_c$, $\epsilon_e$, $k_r$, and $k_a$) determined from the experimental points having been used for the calculation of CRM curves. It can be seen that the calculated curves match the experimental points well.

Generally, results shown in figures 3.9 and 3.10 show that CRM fits almost all exper-
Figure 3.9: The change in surface tension with time and with relative surface area during dynamic cycling of BLES at 10% compression and different concentrations and periodicities: (a) 2 mg/ml and 1 s/cyc; (b) 2 mg/ml and 9 s/cyc; (c) 27 mg/ml and 1 s/cyc; (d) 27 mg/ml and 9 s/cyc. Symbols show the measured surface tension values and lines show the values obtained from CRM.
Chapter 3. Dynamic Surface Tension Model

Figure 3.10: The change in surface tension with time and with relative surface area during dynamic cycling of BLES at 30% compression and different concentrations and periodicities: (a) 2 mg/ml and 1 s/cyc; (b) 2 mg/ml and 9 s/cyc; (c) 27 mg/ml and 1 s/cyc; (d) 27 mg/ml and 9 s/cyc. Symbols show the measured surface tension values and lines show the values obtained from CRM.
imental conditions (high and low concentrations, cycling rates and compression ratios) very well. It can be concluded that the model using only four adjustable parameters is capable of capturing the main features of the dynamic cycling for different conditions. However, there is a slight inconsistency between the model and the experimental results at the high surface tension range in some cases, e.g. figures 3.10(b) and 3.10(d). This disagreement only appears at the highest compression ratio (30%) and the lowest compression rates (9 seconds per cycle). In these cases, the compression is slow, and hence the details of the adsorption and elasticity effects are pronounced near the highest surface tension values. As the area is increased, the surface tension increases due to the film elasticity; however, if the surface tension exceeds the equilibrium value, the surface tension tends to decrease at the same time due to adsorption to the interface. Since the compression is slow in these cases, the surface tension shows some fluctuations near the highest surface tension range. The adsorption in such cases is much quicker than the speed of compression, causing these fluctuations to be very pronounced. Such low compression rates are not really relevant clinically; however, this lack of agreement is considered in more detail below.

The same procedure of extracting the dynamic parameters from every cycle is applied for all 20 cycles in every experimental run. An effective value for each of the parameters is formed by the multi-variable optimization for every cycle. 20 values for each of the four dynamic parameters and the goodness of fit are calculated for every run. From every run, average values are calculated. For a specific condition (concentration, compression ratio, and compression rate), at least three runs were performed. For each of the 36 different experimental conditions considered, the values of the goodness of fit and the four dynamic parameters reported below in figures 3.11 to 3.15 are the average values of the parameter plotted on the ordinate across repeated runs and the standard deviation. The average values of the four dynamic parameters for different conditions are also summarized in table 3.2.
Figure 3.11: The goodness of fit for CRM measured for four different concentrations of BLES (2, 8, 15, 27 mg/ml) at different compression ratios (10%, 20%, 30%) and different cycling conditions (1, 3, 9 s/cycle). The error bars indicate the standard deviation between runs repeated under the same conditions.
The visual judgement of a good fit between experimental points and calculated curves can be quantified by means of a “goodness of fit”. Figure 3.11 compares the values of the goodness of fit from CRM calculated at different experimental conditions. The goodness of fit is defined as:

\[ GF = \sqrt{\sum \left| \frac{\gamma_e - \gamma_m}{\gamma_e} \right|^2} \]  
(3.18)

where \( \gamma_e \) is the experimentally measured surface tension value and \( \gamma_m \) is the predicted surface tension value from CRM. Smaller values for the goodness of fit indicate a better fit. It turns out that CRM fits almost all experimental conditions (high and low concentrations, cycling rates and compression ratios) very well except for a few points at higher compression ratios. The best results for the goodness of fit are at any concentration at low compression ratios and high frequency cycling. These conditions are relevant to clinical practice of high frequency ventilation. The goodness of fit gives an indication of the conditions where CRM works best. This point of the applicability of the model to certain conditions is considered in more detail below.

Figures 3.12 and 3.14 show the calculated elasticity of compression, \( \epsilon_c \), and elasticity of expansion, \( \epsilon_e \), obtained at different experimental conditions. Figure 3.13 and 3.15 show the calculated relaxation coefficient, \( k_r \), and adsorption coefficient, \( k_a \), obtained at different experimental conditions. A detailed inspection of every parameter will follow below. In these four figures, the values reported are the average values across repeated runs and the standard deviation. Asterisks are used in these figures to highlight points where CRM is less sensitive to one or more of the parameters. The quantification of the sensitivity of the model is described in section 3.5.3.2.
Figure 3.12: The elasticity of compression, $\varepsilon_c$, calculated from CRM for four different concentrations of BLES (2, 8, 15, 27 mg/ml) at different compression ratios (10%, 20%, 30%) and different cycling conditions (1, 3, 9 s/cycle). The asterisks show the range of insensitivity (explained in figure 3.17): * indicate points with range of insensitivity bigger than 0.2, ** indicate points with range of insensitivity bigger than 0.5.
Figure 3.13: The relaxation coefficient, $k_r$, calculated from CRM for four different concentrations of BLES (2, 8, 15, 27 mg/ml) at different compression ratios (10%, 20%, 30%) and different cycling conditions (1, 3, 9 s/cycle). The asterisks show the range of insensitivity (explained in figure 3.17): * indicate points with range of insensitivity bigger than 0.2, ** indicate points with range of insensitivity bigger than 0.5.
Figure 3.14: The elasticity of expansion, $\epsilon_e$, calculated from CRM for four different concentrations of BLES (2, 8, 15, 27 mg/ml) at different compression ratios (10%, 20%, 30%) and different cycling conditions (1, 3, 9 s/cycle). The asterisks show the range of insensitivity (explained in figure 3.17): * indicate points with range of insensitivity bigger than 0.2, ** indicate points with range of insensitivity bigger than 0.5.
Figure 3.15: The adsorption coefficient, $k_a$, calculated from CRM for four different concentrations of BLES (2, 8, 15, 27 mg/ml) at different compression ratios (10%, 20%, 30%) and different cycling conditions (1, 3, 9 s/cycle). The asterisks show the range of insensitivity (explained in figure 3.17): * indicate points with range of insensitivity bigger than 0.2, ** indicate points with range of insensitivity bigger than 0.5.
Table 3.2: Summary of the dynamic parameters for BLES at 37°C and 100% R.H. at different conditions. Asterisks show range of insensitivity (explained in figure 3.17): * range of insensitivity bigger than 0.2, ** range of insensitivity bigger than 0.5.

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3.5.3 Discussion

3.5.3.1 Dynamic CRM Parameters

The change of the elasticity of compression, $\epsilon_c$, with concentration, compression ratio and compression rate is shown in figure 3.12. It is expected that the elasticity is an intrinsic property of the lung surfactant preparation that should not change with different experimental conditions. The elasticity of compression did indeed remain almost the same with concentrations above 8 mg/ml. There is also no significant change with compression ratio or compression rate. This agrees with previous studies on BLES with concentrations 0.5 and 2 mg/ml at 20% compression and 3 seconds per cycle [146]. In previous studies of DPPC spread films at compression ratios between 0.1 and 30% and frequencies 0.02, 0.05 and 0.1 Hz, the dilatational elasticities were found to be almost constant for compression ratios higher than 1% and for all frequencies studied [203]. Spread films of of n-dodecyl dimethyl phosphine oxide (DC$_{12}$PO), at compression ratios in the range of 2% to 10% and frequencies in the range of 0.015 to 0.57 Hz, did not show significant change in the dilatational elasticities [166].

Elasticity of compression of BLES was previously evaluated using an elementary procedure [25]. The surface tension-relative area curve during the compression stage was fitted to a fourth order polynomial equation, and the first derivative was used to calculate the dilatational elasticity at the midpoint of the compression. Values obtained from that method are in the range of 100 mJ/m$^2$; these results are lower, but essentially in the same order of magnitude as results presented here. However, it is important to note that the main assumption in that procedure [25] is that the elasticity is the only active parameter during the film compression and that any effects of relaxation during the compression process are negligible.

Other studies of DPPC/SP-B, DPPC/SP-C, DPPC/SP-B/SP-C spread films did not show any dependence of the elasticity on the compression speed [61, 62, 107, 147]. These
results support the concept that more flexible film structures are formed in the presence of surfactant proteins [204]. It can be concluded that the elasticity parameter in CRM is a property of the lung surfactant preparation. In fact, the addition of an inhibitor, e.g. serum, albumin, fibrinogen, or excess cholesterol, to lung surfactant preparations is known to significantly reduce the elasticity of compression of BLES [90].

The change of the relaxation coefficient, $k_r$, with concentration, compression ratio and compression rate is shown in figure 3.13. There is some variability but not a pronounced dependence on surfactant concentration. There is no significant dependence on concentrations at all compression ratios or compression rates except for a few points at the 10% compression. There is a small increase in the relaxation coefficient when the compression ratio is increased from 20% to 30%. This agrees with published results for BLES [25]. The relaxation coefficient seems to depend also on the speed of compression; the higher the frequency, the higher the relaxation rate. This agrees with other studies of BLES [25] and DC$_{12}$PO [166].

The change of the elasticity of expansion, $\epsilon_e$, with concentration, compression ratio and compression rate is shown in figure 3.14. Values are very close to the elasticity of compression. Generally, there is no significant dependence on concentration, compression ratio or compression rate. However, the sensitivity of the model to changes in $\epsilon_e$ of most experiments at 30% compression is low. This leads to less reliable values at these conditions. More details regarding the sensitivity of the model are given in section 3.5.3.2.

The change of the adsorption coefficient, $k_a$, with concentration, compression ratio and compression rate is shown in figure 3.15. There is some variability but not a pronounced dependence on surfactant concentration. However, this variability is reproducible as indicated by the error bars in the figure. The adsorption coefficient seems to increase with the increase of the compression ratio and compression rate. However, the sensitivity of most points at 20% and 30% compression is very low. This leads to less reliable $k_a$ values at these conditions. More details regarding the sensitivity of the model
are given in section 3.5.3.2.

### 3.5.3.2 Sensitivity of CRM

For some experiments reported here, it is noticed that the fitting of CRM to the experimental results is not sensitive to one or more of the four dynamic parameters. To further investigate this point, a detailed sensitivity study was performed. The details of calculating the “range of insensitivity” for each parameter is given in section 3.5.4. Briefly, it is attempted to identify for each parameter the range in which CRM is not highly sensitive. The sensitivity of the model (in terms of the goodness of fit) to changes in the respective parameter is calculated as the percentage change of the goodness of fit divided by the percentage change of the parameter. For example, a sensitivity of 1 means that a relative change of, say 20%, of a specific parameter yields a relative change of the same amount, 20% in this case, of the goodness of fit. A sensitivity cut-off level of 1 is chosen to differentiate between sensitive and insensitive values. The range of percentage change of the parameter in which the sensitivity of the model is below 1 is called here the “range of insensitivity” for a specific parameter. The procedure is applied for each of the four parameters of CRM and the range of insensitivity is calculated for each in all experiments. A summary of these calculations is shown in figure 3.16 and also in table 3.3.

In this figure, the contour lines show the range of insensitivity for each parameter. Higher values indicate that the model is less sensitive to a wider range for this specific parameter. It can be seen that there are similarities between the insensitivity contours of both elasticity of compression and relaxation coefficient and also between both elasticity of expansion and adsorption coefficient.

From these contours, experimental conditions can be identified where CRM is less sensitive to certain parameters. Such information can be very useful as recommendations can be made to what experimental conditions are to be used if there is an interest in
Figure 3.16: Contour lines of the range of insensitivity for each of the parameters: (a) \( \epsilon_c \), (b) \( \epsilon_e \), (c) \( k_r \), (d) \( k_a \), at different periodicities, compression ratios and concentrations. The calculation of the range of insensitivity is explained in figure 3.17.
Table 3.3: Summary of the range of insensitivity of CRM to each of the dynamic parameter (explained in figure 3.17) for BLES at 37°C and 100% R.H. at different conditions.

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<tr>
<th>Conc. (mg/ml)</th>
<th>Comp. (%)</th>
<th>Freq. (s/cyc)</th>
<th>Elasticity of Compression $\epsilon_c$ (mJ/m$^2$)</th>
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a specific parameter. For example, the elasticity of compression, $\epsilon_c$, and the relaxation coefficient, $k_r$, are less sensitive at high concentrations, high compression ratios, and high cycling rates. The fit is also less sensitive to $\epsilon_c$ and $k_r$ at low compression ratios and low cycling rates; at these conditions the parameters $\epsilon_e$ and $k_a$ are more sensitive. The elasticity of expansion, $\epsilon_e$, and the adsorption coefficient, $k_a$, are less sensitive at high concentrations and high compression ratios.

It is important to note that CRM is generally sensitive to all the parameters at any concentration at physiological conditions of 20% compression and 3 seconds per cycle. The model is also sensitive to all the parameters at any concentration at low compression ratios and high frequency cycling. These conditions are relevant to conventional and high frequency ventilation.

Generally, the effect of the concentration on the four dynamic parameters is minimal beyond 8 mg/ml. This is important for clinical therapeutic practice where it is believed that increased concentration will improve the surfactant properties. As shown here, increasing surfactant concentration does improve the dynamic properties only in the low concentration range below 8 mg/ml. Although the dynamic properties of BLES-only preparations do not improve significantly with increasing BLES concentration, the same might not be true in the presence of inhibitors. For example, it was shown before that when albumin is added to BLES, the elasticity value is markedly decreased indicating surfactant inactivation or inhibition. Using ADSA–CSD in combination with CRM, the effect of relevant additives and/or inhibitors could be evaluated at appropriate conditions of concentration, compression rate and compression ratio.

### 3.5.4 Range of Insensitivity

This section explains the calculations of the range of insensitivity for each parameter of CRM. First, the change of goodness of fit (defined in equation 3.18) is recalculated after changing the respective parameter around the calculated value while keeping the rest
Figure 3.17: Calculation of the range of insensitivity for the elasticity of the compression $\epsilon_c$ and the adsorption coefficient $k_a$ of one cycle for 2.0 mg/ml BLES at 20% compression ratio with a periodicity of 9 seconds per cycle: (a) The change of goodness of fit with the change of $\epsilon_c$ around the calculated value while keeping the rest of the parameters ($\epsilon_e$, $k_r$, $k_a$) constant. (b) The change of goodness of fit with the change of $k_a$ around the calculated value while keeping the rest of the parameters ($\epsilon_c$, $\epsilon_e$, $k_r$) constant. (c) The relative change of sensitivity with the relative change of $\epsilon_c$. (d) The relative change of sensitivity with the relative change of $k_a$. The range of insensitivity is the range of percentage change of the parameter in which the sensitivity is below 1.
of the parameters constant. Two examples are shown in figures 3.17(a) and 3.17(b) for one of the cycles of 2.0 mg/ml BLES at 20% compression ratio with a periodicity of 9 seconds per cycle. In figure 3.17(a), the change of goodness of fit is plotted versus the change of one of the parameters (\(\epsilon_c\)) around the calculated value while keeping the rest of the parameters (\(\epsilon_e, k_r, k_a\)) constant. In figure 3.17(b), the change of goodness of fit is plotted versus the change of one of the parameters (\(k_a\)) around the calculated value while keeping the rest of the parameters (\(\epsilon_c, \epsilon_e, k_r\)) constant.

The second step is to calculate the sensitivity of the model (in terms of the goodness of fit) to changes in the respective parameter by calculating the percentage change of the goodness of fit divided by the percentage change of the parameter. For example, for the case of the adsorption coefficient, \(k_a\), the sensitivity of the model with respect to this parameter is

\[
S = \left| \frac{\Delta GF/GF}{\Delta k_a/k_a} \right|
\]  
(3.19)

Samples are shown in figures 3.17(c) and 3.17(d) for the same experiment. In these figure, the relative change of sensitivity is plotted versus the relative change of one of the parameters (\(\epsilon_c\) or \(k_a\)).

The third step is to define the “range of insensitivity” based on the sensitivity calculations. A cut-off level of 1 is chosen to differentiate between sensitive and insensitive values. If the change in the respective parameter yields a sensitivity more than 1, the model is considered sensitive to this parameter at this change level, and vice versa. Hence, the range of insensitivity for this parameter is defined as the range of percentage change of the parameter in which the sensitivity of the model is below 1.

In our example of figure 3.17(c), CRM is found to be insensitive to small changes in this parameter, \(\epsilon_c\), in the range of roughly 98% to 102% of the calculated value for \(\epsilon_c\). This means that the range of insensitivity of the model to the parameter \(\epsilon_c\) is found to be 0.04 in this experiment as shown in the figure. In figure 3.17(d), CRM is found to be insensitive to small changes in this parameter, \(k_a\), in the range of roughly 82% to 133%
of the calculated value for $k_a$. This means that the range of insensitivity of the model to the parameter $k_a$ is found to be 0.51 in this experiment as shown in the figure.

The same procedure is applied for each of the four parameters of CRM and the range of insensitivity is calculated for each in all the experiments. A summary of these calculations is shown in figure 3.16 and also in table 3.3.
Chapter 4

Accuracy and Shape Parameter*

4.1 Introduction

In Chapters 2 and 3 little is known about the accuracy of the surface tension measurements. This is particularly critical in Chapter 3 because the accuracy of the determined mechanical properties of lung surfactant films depends on the accuracy of the measured response of surface tension to changes in surface area. Therefore, high accuracy surface tension measurements are necessary in such studies.

As indicated in Chapter 1, drop shape techniques for surface tension measurement are based on the shape of a pendant drop, sessile drop or captive bubble. Generally, the shape of the drop/bubble depends on the balance between gravity and surface tension. When gravitational and surface tension effects are comparable, then, in principle, one can determine the surface tension from an analysis of the shape of the drop/bubble. Whenever the surface tension effect is much larger than the gravitational effect, the shape tends to become close to spherical in the case of pendant and sessile drops/bubbles. Theoretically, each drop shape corresponds to a certain surface tension value. For well deformed shapes, a slight change in surface tension causes a significant change in the shape. However, for

*Portions of this chapter were previously published in Refs. [205, 206], reproduced with permission.
nearly spherical drop/bubble shapes, a significant change in surface tension causes only a slight change in the shape. Thus, the sensitivity of surface tension to changes in drop shape is low.

Despite the general success and flexibility of ADSA, it is well-documented that, as the drop/bubble shape becomes close to spherical, the performance of all drop shape techniques, including ADSA, deteriorates [64]. The same problem has also been observed by other researchers who are using numerical optimizations for the measurement of interfacial tensions [207–209]. In their study, the surface tension values calculated from large pendant drops of pure liquids are calculated consistently and accurately. However, as the volume of the drop decreases, the surface tension value deviates from the true value.

An illustration of such limitation is shown in figure 4.1, where the surface tension of a pure liquid, OMCTS, is measured in the ADSA–CSD setup while changing the drop volume. The symbols represent the actual measurement, while the straight line represents the literature value. It is clear that the surface tension is correct for the
large drops. However, as the drop volume decreases, the surface tension deviates from the true value. The reason for choosing pure liquids for accuracy studies is that there exists a unique constant value of surface tension for each liquid for all drop sizes; any experimental deviation from that value is attributed to errors or limitations of ADSA. Of course, this becomes more complicated in the case of surfactants, where surface tension changes surface compression and compression rate as shown in Chapters 2 and 3. In fact, two BLES constrained sessile drops of the same concentration and same volume might have very different surface tension values. A serious implication would be clear in case slopes of the surface tension with drop size (specifically drop surface area) are to be calculated. Such slopes are required, for example, to calculate the elasticity of lung surfactant films as shown in Chapter 2 (equation 2.5). A systematic error in the calculations of these slopes should be expected if the surface tension calculated from ADSA deviates systematically (with positive or negative slopes) from the correct value as shown in figure 4.1. Therefore, an independent measure of the quality of the measurement is needed to evaluate the validity of ADSA results using pure liquids. In the literature, there are two different approaches for this problem.

The first approach to quantify the accuracy of the measurements uses a quantitative criterion called “shape parameter” that was introduced to quantify the meaning of “well-deformed” drops and “close-to-spherical” drops [64]. The shape parameter is a dimensionless parameter that expresses quantitatively the difference in shape between a given experimental drop and a spherical shape. A simple approach was used to calculate the difference between the projected area of the drop and a circle with radius $R_0$ (the radius of curvature at the apex of the drop). The mathematical formulation of the shape parameter was

$$P_s = \frac{\left| \frac{2\pi r_0}{\int_0^1 \int_0^{\theta} r dr d\theta} \right| - \pi R_0^2}{\frac{2\pi r_0}{\int_0^1 \int_0^{\theta} r dr d\theta}}$$

(4.1)
Experimentally, the volume of a sessile/pendant drop of pure liquids with known surface tension values was changed from the smallest drop size for which the numerical scheme converges to the largest drop size that a constellation can hold. The surface tension values obtained from ADSA for these drop sizes were compared with the literature value and a shape parameter value was calculated for each drop image. Typically, the error in the surface tension measurement is presented as a function of the shape parameter. A critical shape parameter was defined based on the minimum value of the shape parameter that guarantees an error of less than, say, ±0.1 mJ/m², or any other relative error. The critical shape parameter determines the range of applicability of ADSA. In other words, for a drop shape with a shape parameter larger than the critical value, ADSA performs within an accuracy of ±0.1 mJ/m² [64]. It was found that the critical shape parameter does not depend on Bond number (defined as $\Delta \rho g R_0^2/\gamma$ using $R_0$ as the characteristic length scale in that study) [64].

The second approach reported is a sensitivity test of pendant drops and liquid bridges to measure the interfacial tension [210]. The sensitivity was defined as the change in the projected area of the drop profile due to a small change (5%) in the surface tension. This analysis was applied to synthetic images with pre-defined surface tension values. It was found that the sensitivity of both pendant drops and liquid bridges decreases as the Bond number decreases [210]. The Bond number here was defined using the diameter of the needle used for the pendant drop case or the diameter of the circular holder used for the liquid bridge case as the characteristic length scale. It has to be noted that such sensitivity results will depend on the size of the drop. In the same study, a different approach was used when dealing with experimental images. Briefly, the images of liquid drops of known surface tension values were analyzed using a drop shape technique called TIFA (theoretical image fitting analysis) that fits whole drop images rather than drop profile lines [211, 212]. The surface tension results were compared to the known value for the liquid. A minimum volume was defined based on the minimum value of the drop
volume that guarantees an error of less than 1% in the surface tension measurement. The minimum volume was found to increase for both configurations when the Bond number decreases [210]. One concern with using this approach is that the output of the drop shape technique for surface tension measurement is required to evaluate the quality of the results obtained. The shape parameter, on the other hand, predicts the appropriateness of the subsequent analysis solely based on the acquired drop image.

The idea of comparing a drop shape to a circle (a two dimensional projection of a sphere) used earlier [64] is due to the fact that the shape of sessile/pendant drops or bubbles will be spherical at zero gravity (Bond number of zero). Of course, the sensitivity of any drop shape technique near the zero gravity shape is very limited and approaches zero. Hence comparing the shape of a drop/bubble with the zero gravity shape is an appropriate strategy to evaluate the quality of shape techniques. Therefore, the approach of using a shape parameter [64], i.e. comparing to the case where the sensitivity is effectively zero, is often preferable to using a local sensitivity measure [210] that will change with the drop size. The shape parameter approach is also useful in predicting, \textit{a priori}, the quality of the measurement, i.e. without using the output of the drop shape technique, and hence can be used to design surface tension setups.

For example, when studying lung surfactants using the ADSA–CSD constellation, the surface tension depends both on time and rate of liquid surface area change. Surface area change in turn depends on drop volume change. There are no pure liquids available to mimic the surface tension response of such systems, e.g. near 1 mJ/m$^2$, i.e. a range critical in lung surfactant studies. The shape parameter approach can be used as an experimental guideline to design surface tension setups; if the range of surface tensions to be studied is known, a suitable holder size for the ADSA–CSD setup can be readily selected.

In this chapter, a modified approach to the shape parameter is proposed. The previous definition of shape parameter is based on extracting the value of $R_0$ (the radius of
curvature at the apex of the drop) from the experimental images and comparing projected area of the drop to the circle of that radius \( R_0 \). There are four points that suggest a reworking of this approach. First, this circle (zero gravity shape) may not be the best fit to the experimental profile. Better overall fits may well be obtainable with circles of slightly incorrect \( R_0 \). Second, comparing projected areas might not be the best way to compare the drop profile and the best fit circle. The drop profile and the circle might have similar projected areas and completely different shapes as can be seen from figure 4.2. A more appropriate comparison can be achieved by minimizing the normal distances between the profile and the circle. Third, when dealing with theoretical profiles generated by the Laplace equation for a given value of surface tension and curvature at the drop/bubble apex, experience has shown that the shape parameter values are usually overestimated specially for very small drop volumes. The fourth point is that one would expect that the shape parameter should depend on the Bond number (dimensionless number expressing the ratio of body forces to surface tension forces) contrary to previous findings \[64\] but in agreement with the above sensitivity test \[210\]. It is suspected that using \( R_0 \) as the length scale in the previous shape parameter study \[64\] is not appropriate.

To address these concerns a new definition for the shape parameter is proposed here, in which the experimental drop shape is compared to the circle that fits best all the points.
Chapter 4. Accuracy and Shape Parameter

of the drop profile. The best fit circle is calculated by minimizing the normal distances between the drop profile and the circle. Therefore, the drop profile can be compared to the best fit zero gravity shape (circle in this case); such a circle is not restricted to a specific radius, $R_0$. Thus the search process for the best fit zero gravity shape will include all possible variables, in a similar fashion to how ADSA searches for the best fit profile. This definition will eliminate any dependence on the radius of curvature of the apex. In this case, the circle can have any radius – not only $R_0$ as previously considered [64] – to fit all profile points.

As mentioned earlier, the circle (sphere, in three dimensions) is the zero gravity shape for pendant and sessile drops/bubbles. These zero gravity shapes can be obtained under different conditions. Examples are very low gravity, very high surface tension values, and interfaces between fluids with similar densities. However for other configurations, e.g. liquid bridges or sessile drops formed at the end of a capillary, the shape at zero gravity is not spherical but will depend on the new contact conditions. These drops at zero gravity may assume different shapes including cylindrical, nodoidal, unduloidal and catenoidal. The definition of the shape parameter can be readily extended – in principle – to these configurations by comparing the drop shape to the best fit zero gravity shape that is appropriate to a specific configuration.

In this chapter, a simple strategy to construct the shape parameter is applied to ADSA–CSD in Section 4.2 because of the need for it in Chapters 2 and 3. To understand the relationship between the constrained sessile drop shape and the accuracy of the surface tension measurement, dimensional analysis is used to describe similarity in sessile drop shapes and to express the problem using appropriate dimensionless groups. The proposed shape parameter (in dimensionless form) is found to depend only on two dimensionless groups: the dimensionless volume (drop volume normalized by the cube of the holder radius) and the Bond Number (using the holder’s radius as the length scale).

A set of experiments were performed with pure liquids to illustrate the change of
the critical shape parameter with the Bond number for the two different sessile/pendant setups. To obtain a reasonable range of Bond numbers several pure liquids with known surface tensions and several holder sizes were used as will be shown below. In every experiment, the drop volume is varied and the error in the surface tension measurement is presented as a function of the shape parameter following the same procedure as in literature [64]. A critical shape parameter is defined as the minimum value of the shape parameter that guarantees an error below a stipulated error limit. It will be shown below that the critical shape parameter depends only on the Bond number. Based on the analysis presented here, specific experimental design parameters are recommended for accurate surface tension measurements using constrained sessile drops.

Since it turned out that the very same analysis is applicable to the pendant drops, and since the pendant drop constellation is now the most frequently used surface tension methodology, the analysis for the shape parameter was extended to the pendant drop situation in Section 4.3. The universality of the results is examined is Section 4.4.

4.2 Drop Shapes and Shape Parameter

4.2.1 Dimensional Analysis of Axisymmetric Drop Shapes

Five, and only five, independent parameters affect the shape of the constrained sessile drop: the surface tension of the drop, $\gamma$, the density difference between the drop and the surrounding fluid, $\Delta \rho$, the gravitational acceleration, $g$, the volume of the drop, $V$, and the diameter of the holder, $R_h$. As discussed in Chapter 1 the apparent contact angle neither affects nor defines the drop shape in this constrained sessile drop setup. The drop takes a specific shape depending on only those five independent parameters so that the drop assumes a specific curvature at the apex, $b = 1/R_0$. Hence, it stands to reason that

$$ b = \frac{1}{R_0} = \tilde{\mathfrak{s}}(R_h, V, \gamma, \Delta \rho, g) \quad (4.2) $$
where $R_0$ is the radius of curvature at the apex of the drop. Examples of this relationship are shown below in Section 4.2.3.

Using standard dimensional analysis techniques [213], the parameters of equation 4.2 can be simplified to a set of dimensionless groups. The standard procedure can be summarized briefly as follows: First, equation 4.2 can be rewritten as a relation between parameters in the form

$$f(b, R_h, V, \gamma, \Delta \rho, g) = 0 \quad (4.3)$$

There are 3 fundamental physical units in this equation: mass $m$, length $l$, and time $t$, while there are 6 ($n$) dimensional variables: $b \ [l^{-1}], R_h \ [l^{1}], V \ [l^{3}], \gamma \ [m^{1} \cdot t^{-2}], \Delta \rho \ [m^{1} \cdot l^{-3}], \text{and } g \ [l^{1} \cdot t^{-2}].$ The units of the dimensional quantities are indicated in brackets. The dimensions of the variables in equation 4.3 can be arranged in the form of the following dimensional matrix:

$$
\begin{array}{cccccc}
&t& & & & \\
&m & 0 & 0 & 0 & 1 & 1 & 0 \\
&l & -1 & 1 & 3 & 0 & -3 & 1 \\
t & 0 & 0 & 0 & -2 & 0 & -2 \\
\end{array}
$$

(4.4)

The rank of this dimensional matrix is $r = 3$. Thus, there are only $n - r = 3$ independent dimensionless combinations (parameters), denoted $\pi_1, \pi_2, \pi_3$, and equation 4.3 can be re-expressed as

$$f(\pi_1, \pi_2, \pi_3) = 0 \quad (4.5)$$

At this point, any 3 ($=r$) of the variables can be selected as “repeating variables”, that will be repeated in all of the dimensionless parameters. In this case, we choose $R_h$ (a characteristic length scale), $\gamma$, and $g$ as the repeating variables. Although other choices would result in a different set of dimensionless parameters, other complete sets can be obtained by combining the ones already computed. Therefore, any choice of the repeating variables is satisfactory.
Each dimensionless parameter is formed by combining the three repeating variables with one of the remaining variables. For example, let the first dimensional product be taken as

$$\pi_1 = b \cdot R_h^a \cdot \gamma^b \cdot g^c$$  \hspace{1cm} (4.6)

The exponents $a$, $b$, and $c$ are obtained from the requirement that $\pi_1$ is dimensionless. This requires:

$$m^0 \cdot l^0 \cdot t^0 = (l^{-1}) \cdot (l^1)^a \cdot (m^1 \cdot t^{-2})^b \cdot (l^1 \cdot t^{-2})^c$$  \hspace{1cm} (4.7)

By equating the indices, we obtain $a = 1$, $b = 0$, $c = 0$, so that

$$\pi_1 = b \cdot R_h^1 \cdot \gamma^0 \cdot g^0 = b \cdot R_h = \frac{R_h}{R_0} = b_d$$  \hspace{1cm} (4.8)

A similar procedure gives:

$$\pi_2 = V \cdot R_h^a \cdot \gamma^b \cdot g^c = \frac{V}{R_h^3} = V_d$$  \hspace{1cm} (4.9)

$$\pi_3 = \Delta \rho \cdot R_h^a \cdot \gamma^b \cdot g^c = \frac{\Delta \rho g R_h^2}{\gamma} = Bo$$  \hspace{1cm} (4.10)

Therefore the dimensionless representation of equation 4.2 is of the form

$$b_d = \frac{R_h}{R_0} = f\left(\frac{V}{R_h^3}, \frac{\Delta \rho g R_h^2}{\gamma}\right) = f(V_d, Bo)$$  \hspace{1cm} (4.11)

Equation 4.11 shows that the dimensionless curvature, $b_d$, (the apex curvature normalized by the holder’s radius) of the drop apex depends only on two dimensionless groups. The first one is the dimensionless volume, $V_d$, which is the drop volume normalized by the cube of the holder’s radius. The second one is effectively the Bond Number, $Bo$, using the holder’s radius as the length scale for this case. Examples of this relationship are shown below in Section 4.2.3.
4.2.2 Shape Parameter

The proposed definition of the shape parameter for the ADSA-CSD setup is a comparison of the drop profile with the circle that best fits all the points of the drop profile. Such definition will eliminate any dependency on the radius of curvature of the apex. In this case, the circle can have any radius – not only $R_0$ as previously used – to fit all profile points.

A simple and computationally inexpensive algebraic fit [214] can be used to find the best fit circle for a number of points (i.e., digitized drop image). Here, the sum of squares of algebraic distances is minimized

$$F(a, b, R) = \sum_{i=1}^{n} \left[ (x_i - a)^2 + (z_i - b)^2 - R^2 \right]^2 = \sum_{i=1}^{n} \left( e_i + Ax_i + Bz_i + C \right)^2 \quad (4.12)$$

where $a$ is the x-axis location for the circle center point, $b$ is the z-axis location for the circle center point, $R$ is the circle radius, $e_i = x_i^2 + z_i^2$, $A = -2a$, $B = -2b$, and $C = a^2 + b^2 - R^2$. Now differentiating $F$ with respect to $A$, $B$, $C$ yields a system of linear equations that can be solved to yield $A$, $B$, $C$, and hence $a$, $b$, $R$ can be computed. Other algebraic fit methods exist to calculate the circle that best fits a list of points [215, 216]. Such methods were also tested in this context, and the results of the calculated shape parameter yielded the same result.

Once the best fit circle is determined, the normal distances between every point in the drop profile and the best fit circle can be simply calculated and used to define the shape parameter as

$$P = \sqrt{\frac{1}{n} \sum_{i=1}^{n} \left[ \sqrt{(x_i - a)^2 + (z_i - b)^2 - R^2} \right]^2} \quad (4.13)$$

To eliminate the effect of the size of the drop, it is desirable to define the shape parameter in dimensionless form. A non-dimensional form can be calculated by normalizing
the value calculated above with the radius of the best fit circle

\[ P_c = \frac{1}{R} \sqrt{\frac{1}{n} \sum_{i=1}^{n} \left( \sqrt{(x_i - a)^2 + (z_i - b)^2} - R \right)^2} \]  (4.14)

The main conclusion that can be drawn from the previous section is that all drop shapes depend on only two dimensionless groups, i.e. the dimensionless drop volume and the Bond number. Since the shape parameter depends only on the drop shape, we postulate that the shape parameter, in its dimensionless form as defined in equation 4.14, depends on the same two dimensionless groups, in a similar fashion as equation 4.11.

\[ P_c = \mathcal{F} \left( \frac{V}{R_h^3}, \frac{\Delta \rho g R^2}{\gamma} \right) = \mathcal{F}(V_d, Bo) \]  (4.15)

This means that every drop shape, and it’s associated shape parameter, depends only on \( V_d \) and \( Bo \). Similar shapes will have similar shape parameters. Examples of this relationship are shown below in Section 4.2.3.

This should not be understood to mean that \( P_c \) is equivalent to \( b_d \). \( P_c \) is the shape parameter as calculated from the digitized drop profile using equation 4.14. \( P_c \) is an indication of the difference in shape between a given experimental drop profile and the best fit circle. On the other hand, \( b_d \) is the dimensionless curvature at the drop apex and is used here as an indication of the drop shape.

### 4.2.3 Sample Constrained Sessile Drops

The relationship between the curvature of the drop apex and the independent parameters affecting the shape of the constrained sessile drop as shown in equation 4.2 is further illustrated here. Figure 4.3 shows the change of the curvature at the constrained sessile drop apex with the drop volume for different holder sizes (outside radius of 3 and 6 mm) and surface tension values (18, 36, and 72 mJ/m²). These results were obtained using...
Figure 4.3: The change of the apex curvature of a constrained sessile drop with surface tension and drop volume for two different holder sizes: (a) outer radius of 3 mm, and outer radius of (b) 6 mm.
the Axisymmetric Liquid Fluid Interface (ALFI) program [9], that predicts the shape of a drop/bubble through numerical integration of the Laplace equation. The inputs for the ALFI program are the apex curvature, $b$, and the capillary constant, $c = \Delta \rho g / \gamma$. The outputs are the generated drop profile, drop surface area, drop volume, $V$, and the contact angle at the end of the drop profile. The end condition for the integration is the radius of the holder, $R_h$.

For the same holder size and the same surface tension value, as the drop volume increases, the curvature at the drop apex increases initially, then decreases when passing through the 90° contact angle. The same curvature can be reached twice on every curve, one time for a small drop volume and another for a large drop volume. There exists a maximum drop volume and a maximum curvature at the drop apex for a given holder size and surface tension value. The maximum curvature at the drop apex corresponds to the case of the 90° contact angle. For the same holder size, as the surface tension increases, the maximum volume and the maximum apex curvature increase. As the holder size increases, the range of possible drop volumes increases and the range of possible curvatures at the drop apex decreases.

The dimensional analysis in Section 4.2.1 (equation 4.11) shows that the dimensionless curvature, $b_d$, of the constrained sessile drop apex depends only on two dimensionless groups: the dimensionless volume, $V_d$, and the Bond Number, $Bo$. Figure 4.4 shows the predicted change of the dimensionless curvature at the constrained sessile drop apex with the dimensionless drop volume for different Bond number values (1.22, 2.44, and 4.89). This figure is considered a general map of the relationship of the three dimensionless groups for the constrained sessile drop problem. The effect of the holder size is effectively integrated inside every one of the dimensionless groups.

For the same Bond number value, as the dimensionless drop volume increases, the dimensionless curvature increases initially, then decreases. The same dimensionless curvature can be obtained twice on every curve, one time for a small dimensionless drop
Figure 4.4: The change of the dimensionless apex curvature of a constrained sessile drop with Bond number and dimensionless drop volume. The same relationship can be obtained using any holder size.

There exists a maximum dimensionless drop volume and a maximum dimensionless curvature at the drop apex for a given Bond number value. As the Bond number decreases, the maximum dimensionless volume and the maximum dimensionless apex curvature increase.

To further investigate similarity in drop shapes, figure 4.5 shows the predicted drop profile shapes for different conditions. Figure 4.5(a) shows the shape of the constrained sessile drop generated using ALFI for the case of surface tension of 6 mJ/m², holder radius of 3 mm, and apex curvature of 0.51 cm⁻¹. This is equivalent to a Bond number of 14.67 and a dimensionless curvature of 0.153. For the given curvature at the apex, there exist two drop volumes that can satisfy these conditions. The dimensionless volumes for the two drops are 0.412 and 1.598. The large volume corresponds to the case where the maximum drop diameter is larger than the holder diameter. This case is indicated with solid symbols in the figure. The other case of small volume is indicated with open
Figure 4.5: The shape of a constrained sessile drop for different holder sizes and surface tension values. Bond number is indicated on top of every figure. The open symbols indicate a drop profile with a small volume, while the solid symbols symbols that with a large volume. The lines indicate the best circle that fit the profile.
symbols. The continuous lines indicate the best fit circles that are fitted to all the drop profile points; these are the circles used in the definition of the shape parameter as shown in equation 4.14. It is clear that the large volume drop is well deformed, but the small volume can be fitted to a circle and hence will not allow accurate surface tension measurement with ADSA.

A case of the very same Bond number is considered in figure 4.5(b). In this case, the drop shape is generated for a surface tension of 54 mJ/m$^2$, holder radius of 9 mm, and apex curvature of 0.17 cm$^{-1}$. This is equivalent to the same Bond number of 14.67 and the same dimensionless curvature of 0.153. For the given curvature at the apex, there exist two drop volumes that can satisfy these conditions. The dimensionless volumes for the two drops are exactly the same as before, i.e. 0.412 and 1.598. It is clear that the drop shapes in figure 4.5(b) are similar to those in figure 4.5(a). The only difference is the scaling factor. Therefore, it is expected that the shape parameter should be exactly the same for both cases. Furthermore, ADSA should treat them similarly, i.e. ADSA will process the well deformed drops (solid symbol) satisfactorily and will not work accurately with the small volume drops (open symbols).

A higher surface tension case is considered in figure 4.5(c). In this case, the drop shape is considered for the case of surface tension of 216 mJ/m$^2$, holder radius of 3 mm, and apex curvature of 2.1 cm$^{-1}$. This is equivalent to a Bond number of 0.41 and a dimensionless curvature of 0.63. For the given curvature at the apex, there exist two drop volumes that satisfy these conditions. The dimensionless volumes for the two drops are 0.613 and 8.734. It is clear that both drops can be fitted well to a circle and hence will not work accurately with ADSA. A similar Bond number case is considered in figure 4.5(d). In this case, surface tension is 6 mJ/m$^2$, holder radius is 0.5 mm, and apex curvature is 12.6 cm$^{-1}$. This is equivalent to the same Bond number of 0.41 and the same dimensionless curvature of 0.63. The dimensionless volumes for the two drops are exactly the same as before, i.e. 0.613 and 8.734. It is clear that the drop shapes in
Figure 4.6: The change of the shape parameter of a constrained sessile drop with Bond number and dimensionless drop volume. The same relationship can be obtained using any holder size.

Following the similarity between equations 4.11 and 4.15, a map similar to that of figure 4.4 can be readily derived. Figure 4.6 shows the change of the dimensionless shape parameter with the dimensionless drop volume for different Bond number values (1.22, 2.44, and 4.89). For the same Bond number value, as the dimensionless drop volume increases, the value for $P_c$ increases indicating more deformed drops. There exists a maximum value for $P_c$ for a given Bond number value. According to this definition of the shape parameter, the minimum value for $P_c$ is always zero. This indicates that very small drops are not appropriate for surface tension measurement even for drops with small surface tension values on large holders. This figure is considered a general map of the relationship of the three dimensionless groups for the constrained sessile drop problem. The same relationship can be obtained using any holder size.

It can be observed that forming the biggest drop possible on a specific holder size is
not always sufficient to obtain accurate results from ADSA. This is clear from the large volume drops at low Bond number values as shown in figures 4.5(c) and 4.5(d). Although the drop volume is large, the drop shape is still very close to a sphere preventing ADSA from calculating accurate results. As the Bond number increases, the drop shape becomes more deformed at large volume. Nevertheless, even at high Bond number values, the drop volume has to be large enough to produce a well deformed drop.

At this point, it is appropriate to assume that at any Bond number, there is a critical shape parameter above which the drop shape is considered well deformed. This critical shape parameter corresponds to a critical volume that the drop has to exceed to be well deformed. This critical shape parameter is expected to increase with the decrease in Bond number. To further investigate this point, a set of experiments were performed to illustrate the change of the critical shape parameter with the Bond number as illustrated below.

4.2.4 Experimental Details

4.2.4.1 Materials and Methodology

Octamethylcyclotetrasiloxane (OMCTS) $[\text{C}_8\text{H}_{24}\text{O}_4\text{Si}_4]$ was purchased from Sigma-Aldrich Co. (cat. 74811) with purity $\geq 99.0\%$, Diethyl phthalate (DEP) $[\text{C}_{12}\text{H}_{14}\text{O}_4]$ from Sigma-Aldrich Co. (cat. 524972) with purity $\sim 99.5\%$, and Water from Sigma-Aldrich Co. (cat. 95286). These three liquids were chosen because they have adequate vapour pressure and cover a wide range of surface tension. OMCTS, DEP, and water have surface tensions of approximately 18, 36, and 72 mJ/m$^2$, respectively.

A schematic diagram of the experimental setup for ADSA–CSD is shown in Figure 4.7. Details of the setup and how ADSA works were given before in Chapter 2. Two holder sizes were used in this study, outer diameters of 6.55 and 7.86 mm. To ensure consistency, the quality of the acquired images (or more specifically, the number of the pixels of the
extracted drop profile) was maintained to be as constant as possible for all experiments. This is done by using different magnifications for different holder sizes. If the same magnification were used for all holders, the drops on the smaller holders would have fewer pixels at the interface and the calculated surface tension values would be less accurate.

4.2.4.2 Experimental Procedure

A large drop of the liquid (roughly 80 $\mu$l) was formed on the stainless steel pedestal which is connected to the syringe and the stepper motor. All experiments were performed at room temperature of 24$^\circ$C. The constrained sessile drop is covered with a glass cuvette to isolate the drop from the environment and to prevent contamination. Several images were acquired and analyzed by ADSA, providing the surface tension of the pure liquid. The drop volume was then decreased very slowly with the motor-controlled syringe and then increased again. The rate of liquid withdrawal and addition was fixed at 0.6 $\mu$l/s, for all experiments. During the volume reduction and enlargement, images were acquired at the rate of two images per second and saved for further analysis.

All acquired images were analyzed by ADSA, providing a value for the surface tension, $\gamma$, the drop surface area, $A$, the drop volume, $V$, and the curvature at the apex, $b$. Figure 4.8 shows a typical result of ADSA for a constrained sessile drop of OMCTS formed on top of a holder with outer diameter of 7.86 mm. This is the same experiment shown previously in figure 4.1. As mentioned earlier, there exists a unique constant value of surface tension for each liquid, any experimental deviation from that value is
Figure 4.8: The change of surface tension, drop surface area, drop volume, and the curvature at drop apex with time of a constrained sessile drop of OMCTS formed on top of a holder with outer diameter of 7.86 mm.
attributed to errors or limitations of ADSA.

4.2.5 Results and Discussion

The surface tension values of large drops (at the beginning and at the end of every run) are constant, i.e. they do not change with drop size, and they agree with the literature value. However, values calculated from small drops are not constant and show irregular trends, i.e. not just random errors, as shown in figure 4.8. All acquired images were analyzed to calculate the shape parameter values according to equation 4.14. To calculate the critical shape parameter for every experiment, the procedure proposed previously [64] was followed. Briefly, the shape parameter values of every run are plotted against the calculated surface tension for this run. The critical shape parameter is the value below which the error in the calculated surface tension value exceeds a specific limit, usually set at ±0.1 mJ/m².

Figure 4.9 shows the error in surface tension measured by ADSA as a function of the shape parameter for different drop sizes of OMCTS formed on a holder with outer diameter of 7.86 mm. This case is equivalent to a Bond number of 7.91. For a maximum error of ±0.1 mJ/m², the critical shape parameter is 0.018. It is to be noted that for large drop sizes (large shape parameter values), the measured surface tension is accurate and consistent. It is also important to note that the overall error in surface tension for this case did not exceed ±1 mJ/m² for almost all drops in this run, except for very small values of shape parameter below 0.005 where the drop is very small and is essentially a spherical cap.

It was noted in figure 4.9 that there is a specific trend for the direction (positive or negative) of the error in the surface tension measurements. Similar trends were also noted in other cases. Figure 4.10(a) shows the error in surface tension of OMCTS formed on a holder with outer diameter of 6.55 mm, while figure 4.10(b) shows the error in surface tension of DEP formed on a holder with outer diameter of 7.86 mm. Both figures show
Figure 4.9: The error in surface tension measured by ADSA as a function of the shape parameter for different drop sizes of a constrained sessile drop of OMCTS formed on top of a holder with outer diameter of 7.86 mm. For a maximum error of $\pm 0.1 \text{ mJ/m}^2$, the critical shape parameter is 0.018.

similar – but not exactly the same – trends to that of figure 4.9. To further clarify this, a series of synthetic profiles were generated from ALFI at the same conditions (same surface tension, holder size, drop volumes) and were analyzed with ADSA. It was noted that the error in this case has no tendency to take positive or negative values, i.e. there are no systematic deviations from the correct value. Therefore, it can be concluded that the systematic deviations observed experimentally is not an artifact of the optimization procedure used. The deviations might be caused by the quality of the images and the related optical conditions.

The same analysis was applied to experiments using the other two liquids, diethyl phthalate (DEP) and water, using the two holders with outer diameters of 6.55 and 7.86 mm. All the results are summarized in Table 4.1. The first two columns of the table list the holder size and the liquid used for every experiment. The third column lists the presumably correct surface tension values as found in the literature and/or previous
Figure 4.10: The error in surface tension measured by ADSA as a function of the shape parameter for different drop sizes of a constrained sessile drop of: (a) OMCTS formed on top of a holder with outer diameter of 6.55 mm, and (b) DEP formed on top of a holder with outer diameter of 7.86 mm. For a maximum error of ±0.1 mJ/m², the critical shape parameters are 0.026 and 0.027, respectively.
Table 4.1: Summary of results of constrained sessile drop experiments using different liquids and different holder sizes.

<table>
<thead>
<tr>
<th>Holder (mm)</th>
<th>Liquid</th>
<th>γ (mJ/m²)</th>
<th>Bo</th>
<th>( \gamma_{\text{measured}} ) (mJ/m²)</th>
<th>( P_{\text{crit}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.20</td>
<td>Water</td>
<td>72.39</td>
<td>0.60</td>
<td>71.023 ± 0.011</td>
<td>70.992 ± 0.012</td>
</tr>
<tr>
<td>6.55</td>
<td>Water</td>
<td>72.39</td>
<td>1.46</td>
<td>72.361 ± 0.015</td>
<td>72.321 ± 0.017</td>
</tr>
<tr>
<td>7.86</td>
<td>Water</td>
<td>72.39</td>
<td>2.10</td>
<td>72.354 ± 0.017</td>
<td>72.313 ± 0.009</td>
</tr>
<tr>
<td>6.55</td>
<td>DEP</td>
<td>36.95</td>
<td>3.18</td>
<td>36.861 ± 0.013</td>
<td>36.865 ± 0.013</td>
</tr>
<tr>
<td>7.86</td>
<td>DEP</td>
<td>37.95</td>
<td>4.58</td>
<td>36.936 ± 0.006</td>
<td>36.913 ± 0.008</td>
</tr>
<tr>
<td>6.55</td>
<td>OMCTS</td>
<td>18.20</td>
<td>5.41</td>
<td>18.110 ± 0.009</td>
<td>18.137 ± 0.006</td>
</tr>
<tr>
<td>7.86</td>
<td>OMCTS</td>
<td>18.20</td>
<td>7.91</td>
<td>18.177 ± 0.006</td>
<td>18.206 ± 0.004</td>
</tr>
</tbody>
</table>
work [217–219]. The fourth column lists the Bond number for every experiment; a range of Bond numbers between 0.60 and 7.91 is covered in this study. The following three columns contain the calculated surface tension value (from the largest drop sizes) and the accuracy of the calculation; more than 60 drops were used in the calculation of the average value and the standard deviation. The last three columns contain the measured critical shape parameter for every experimental run. These values were measured in a similar procedure used in figure 4.9.

Inspection of table 4.1 shows that for all systems, except the first one listed, there is excellent agreement from run to run, as well as with the standard values of column 3. It is also noted that the differences from run to run could be due to ambient temperature changes of the order of 0.1°C. The consistency from run to run and the accuracy of each run are generally less for the first system, and the agreement with the standard values is much poorer, out of the stipulated error range of ±0.1 mJ/m². A critical shape parameter does not exist for these cases. The reason of this difference in pattern is discussed in detail below.

Figure 4.11 shows the change of the critical shape parameter as a function of the Bond number for all the experiments of this study. In other words, the critical shape parameter values for every run obtained in a similar fashion to figure 4.9 and summarized in the last three columns of table 4.1 are used to calculate an average critical shape parameter value for every Bond number as shown in figure 4.11.

Therefore, for a specific liquid, i.e. a specific surface tension value, and a specific holder size, there exists a critical shape parameter value below which the error in the calculated surface tension value exceeds a specific limit. If we use the same liquid with a larger holder size, the drop shapes become more deformed and the calculated critical shape parameter value is lower. A similar case exists when a different liquid with a smaller surface tension value is used with the same holder size. The drop shapes become more deformed and the calculated critical shape parameter value is lower. A larger holder
Figure 4.11: The critical shape parameter as a function of the Bond number. The experimental points were fitted to a linear function (continuous line).

or a liquid with a smaller surface tension value leads to a larger Bond number (using the holder’s radius as the length scale).

As expected, figure 4.11 shows that the critical shape parameter decreases with increasing Bond number. The critical shape parameter is equivalent to a critical dimensionless volume (the drop volume normalized by the cube of the holder’s radius) that the drop has to exceed to allow for accurate measurement of the surface tension. This can also be understood from figure 4.6 which defines the relationship between the shape parameter, dimensionless volume and Bond number. The critical shape parameter is one point on every curve below which the drop shape is indistinguishable from the zero gravity shape (circle in this case). For high Bond numbers (a low surface tension liquid and/or a large holder), it is expected that small drop volumes are sufficient to have well deformed shapes. On the other hand, for low Bond numbers (a high surface tension liquid and/or a small holder), it is expected that very large drop volumes are needed to make sure that the drop is well deformed.
It is expected that as the Bond number increases, the critical shape parameter will decrease approaching the value of zero as the Bond number approaches infinity. On the other hand, as the Bond number decreases and approaches zero, the critical shape parameter will increase dramatically. However, it was found in this study that critical shape parameters cannot be established for Bond numbers below one, i.e. when the diameter of holder becomes small and approaches zero. This is due to the fact that the largest drop that can be formed on a given holder size is not big enough to calculate an accurate surface tension value.

The relationship between the critical shape parameter and the Bond number can be considered as a universal guide. Therefore, it was attempted to fit these points in this range with a straight line as shown in the figure. Of course, on a wider range, it is expected to have an exponential form. However, using a straight line in the experimental range here seems sufficient. The best fit line is plotted in figure 4.11 as a continuous line. Such a relationship is only applicable for a desired error of ±0.1 mJ/m² in the measured surface tension. Changing this value for the desired error to a higher or a lower value would change the location of this curve downwards or upwards, respectively. However, such change in the stipulated error will not have any effect on the shape of the curve.

The relation between the critical shape parameter and the Bond number should be universal for this specific constrained sessile drop constellation. To further illustrate this point, an additional experiment was performed using a fourth liquid, hexadecane (with surface tension of 27 mJ/m²), and the holder with outer diameter of 7.86 mm. The Bond number is 4.3 for this experiment, and the critical shape parameter is expected to be 0.0331 from figure 8. The hexadecane experiment was repeated three times and the measured critical shape parameter was found to be 0.034±0.0014. This value agrees with the expected value and fits well with the other points shown in figure 4.11.

The relationship between the critical shape parameter and the Bond number can be used in the design of a constrained sessile drop experiment for surface tension measure-
Chapter 4. Accuracy and Shape Parameter

Through the above relationship, we know that a high Bond number is always preferable and will facilitate more accurate surface tension measurement. A high Bond number is equivalent to a small surface tension value and/or a large holder size. If a rough approximation of the surface tension is known, the right holder size can be easily selected to produce accurate measurement.

For example, based on the calculations presented here and assuming a density difference of 1000 kg/m³ and gravity of 9.80 m/s², designing an ADSA–CSD experiment to measure surface tension values in the range of 0.5 to 38 mJ/m² would require a minimum holder diameter of 4 mm to ensure a maximum error of ±0.1 mJ/m² throughout. Such range of surface tension values is of interest in the case of lung surfactant studies, or compressed soluble or insoluble films. Based on this analysis, results of Chapter 3 can be confirmed to be within the ±0.1 mJ/m² error range. It has to be noted that using a smaller holder for this case will cause a larger error near the high end of this surface tension range. Generally, it is advisable to make the drop as large as possible to guarantee accurate results especially near the high end of this range. Another example is designing an experiment to measure surface tension in the range of 15 to 72 mJ/m², e.g. common liquids, which would require a minimum holder diameter of 6 mm. Based on this analysis, results of Chapter 2 can be confirmed to be within the ±0.1 mJ/m² error range. These rough examples serve as a guide for selecting the right holder size to produce accurate measurement, if a rough approximation of the surface tension is known. It is important to emphasize that it is the largest surface tension values that dictate a minimum holder size.

4.3 Pendant Drop Shapes

In the pendant drop setup, a drop is formed at the end of a needle. Alternatively, a small flat circular holder (inverted pedestal) with a sharp-knife edge is used instead of
the capillary \[ \text{[59, 64]} \] as indicated in Chapter \[ 1 \]. The dimensional analysis of Section \[ 4.2.1 \] developed for the constrained sessile drop case is still applicable to the pendant drop case because the same five independent parameters affects the shape of both the pendant drop as well as the constrained sessile drop (Section \[ 4.2.1 \]). The definition of the shape parameter presented in Section \[ 4.2.2 \] can be also used for the pendant drop case, since they both share the same zero gravity shape (sphere). This would not be appropriate, e.g., in the case of a liquid bridge.

This section follows the same logic as that of Section \[ 4.2 \]. The relationship between the curvature of the drop apex and the independent parameters affecting the shape as shown in equation \[ 4.2 \] is further illustrated here for the pendant drop. The change of the curvature, \( b \), at the pendant drop apex with the drop volume for different holder sizes (outside diameter of 3 and 6 mm) and surface tension values (18, 36, and 72 mJ/m\(^2\)) is shown in figure \[ 4.12 \]. These results were obtained using ALFI as mentioned before in the case of the constrained sessile drop.

For a given holder size and given surface tension value, as the drop volume increases, the curvature at the drop apex increases initially, then decreases in some cases. There exists a largest drop volume, \( V \), and a largest apex curvature, \( b \), for a given holder size and a given surface tension value. Drop profiles can not be generated beyond these values. This will be discussed in more detail below. For a given holder size, when a liquid with a higher surface tension value is used, the largest volume increases and the largest apex curvature decreases. For larger holders, the range of possible drop volumes is larger and the range of possible curvatures at the drop apex is smaller.

The dimensional analysis shown in Section \[ 4.2.1 \] (equation \[ 4.11 \]) shows that the dimensionless curvature, \( b_d \), of the pendant/sessile drop apex depends only on two dimensionless groups: the dimensionless volume, \( V_d \), and the Bond Number, \( Bo \). The predicted change of the dimensionless curvature, \( b_d \), of a pendant drop with the dimensionless drop volume for different Bond number values (1.22, 2.44, and 4.89) is shown in figure \[ 4.13 \].
Figure 4.12: The change of the apex curvature of a pendant drop with surface tension and drop volume for two different holder sizes: (a) outer radius of 1.5 mm; (b) outer radius of 3 mm.
Figure 4.13: The change of the dimensionless apex curvature, $b_d$, of a pendant drop with Bond number, $Bo$, and dimensionless drop volume, $V_d$. The Bond number, $Bo = \frac{\Delta \rho g R_h^2}{\gamma}$, is the ratio of body forces (gravitational) to surface tension forces using the holder’s radius as the length scale.
These Bond numbers correspond to the surface tension values of 72, 36, and 18 mJ/m² using a holder with radius of 3 mm (same cases considered in figure 4.12(b)). Figure 4.13 is considered a general map of the relationship of the three dimensionless groups for the pendant drop problem (similar to figure 4.4 for the constrained sessile drop case). The effect of the holder size is integrated inside every one of the dimensionless groups. For example, a Bond number of 1.22 is equivalent to the two arbitrary chosen cases of: (1) \( R_h = 1.5 \text{ mm} \) and \( \gamma = 18 \text{ mJ/m}^2 \), shown in figure 4.12(a), and (2) \( R_h = 3 \text{ mm} \) and \( \gamma = 72 \text{ mJ/m}^2 \), shown in figure 4.12(b).

It can be seen from figure 4.13 that there exists a largest dimensionless drop volume, \( V_d \), and a largest dimensionless curvature, \( b_d \), for a given Bond number value. Drop profiles can not be generated beyond these values. This will be discussed in more detail below. As the Bond number increases (i.e. a larger holder and/or a liquid with smaller surface tension value is used), the largest dimensionless volume decreases and the largest dimensionless apex curvature increases.

Figure 4.14 shows four examples of the predicted drop profile shapes for different conditions. Figure 4.14(a) shows a pendant drop with \( \gamma = 10.0 \text{ mJ/m}^2 \), \( R_h = 1.0 \text{ mm} \), \( b = 13.2 \text{ cm}^{-1} \), \( Bo = 1.0 \), \( b_d = 1.32 \), and \( V_d = 5.26 \). Figure 4.14(b) shows a pendant drop with \( \gamma = 40.0 \text{ mJ/m}^2 \), \( R_h = 2.0 \text{ mm} \), and \( b = 6.6 \text{ cm}^{-1} \), and hence has exactly the same \( Bo = 1.0 \), \( b_d = 1.32 \), and \( V_d = 5.26 \) as figure 4.14(a). The continuous lines indicate the best fit circle that are fitted to all the drop profile points; this circle is used in the definition of the shape parameter as shown in equation 4.14. It is apparent that both drops are well deformed. The only difference is the scaling factor. Therefore, ADSA should treat them similarly, i.e. ADSA is expected to process these well deformed drops satisfactorily.

A lower surface tension case is considered in figure 4.14(c). In this case, the pendant drop shape is considered for the case of \( \gamma = 1.0 \text{ mJ/m}^2 \), \( R_h = 1.0 \text{ mm} \), \( b = 25.3 \text{ cm}^{-1} \), \( Bo = 10.0 \), \( b_d = 2.53 \), and \( V_d = 0.60 \). Figure 4.14(d) shows a pendant drop with \( \gamma = \)}
Figure 4.14: The shape of a pendant drop for different holder sizes and surface tension values. Bond number is indicated on top of every figure. The symbols indicate the drop profile, and the lines indicate the best circle that fit the profile.
10.0 mJ/m², \( R_h \) of 3.16 mm, and \( b \) of 8.0 cm\(^{-1} \), and hence has exactly the same \( Bo \) of 10.0, \( b_d \) of 2.53, and \( V_d \) of 0.60 as figure 4.14(c). It is clear that both drop can be fitted to a circle fairly closely and hence will not work accurately with ADSA. It is noted that constrained sessile drops at high Bond numbers are expected to be well deformed as shown in figures 4.5(a) and 4.5(b) however, it seems that this is not true for the pendant drop case as shown in figures 4.14(c) and 4.14(d) where drops at high Bond numbers are very close to spherical. This point is discussed later in more detail.

Following the similarity between equations 4.11 and 4.15, a map similar – but not equivalent – to that of figure 4.13 can be readily derived. Figure 4.15 shows the change of the dimensionless shape parameter with the dimensionless drop volume for different Bond number values (1.22, 2.44, and 4.89). For the same Bond number value, as the dimensionless drop volume increases, the value for \( P_c \) increases indicating more deformed drops. There exists a largest dimensionless volume and a largest value for \( P_c \) for a given Bond number value. According to this definition of the shape parameter, the minimum
value for $P_c$ is always zero, similar to the constrained sessile drop case. This indicates that very small drops, with small dimensionless volumes, are not appropriate for surface tension measurement even for drops with small surface tension values on large holders. Figure 4.15 is a general map of the relationship of the three dimensionless groups for the pendant drop problem.

The fact that there exists a largest dimensionless volume for a given Bond number value is logical and expected: The pendant drop may be successively increased in size by adding small amounts of liquid until, at some limiting volume, the drop becomes unstable and a large portion falls away. The growth and the general conditions that lead to the instability of a pendant drop were studied before by Padday and Pitt [71]. In that study, the case of the volume-radius limited pendant drop is equivalent to the case considered here where the volume of the drop is controlled and the holder used has a fixed radius that is completely wetted.

Padday and Pitt [71] studied the criteria of critical stability of axisymmetric menisci in a gravity field and presented a useful form of the critical volume data of the volume-radius limited pendant drop in terms of $V/X^3$ and $X/k$, where $V$ is the drop volume, $X$ corresponds to the radius of the holder, and $k$ is a form of the capillary constant defined as $(\gamma/\Delta \rho g)^{1/2}$. Therefore, $V/X^3$ is equivalent to the dimensionless volume $V_d$ considered here, and $X/k$ is basically the square root of the Bond number $Bo$. The relationship between the largest (i.e. critical [71] or maximum allowable) dimensionless volume and the Bond number is recalculated here using ALFI and plotted in figure 4.16(a). Each point in figure 4.16(a) is equivalent to the end point of the appropriate curve in figure 4.13. The analysis was repeated for a wide range of Bond numbers ranging from $10^{-4}$ to $10^2$. The points are in excellent agreement with those presented by Padday and Pitt [71].

The maximum dimensionless volume as a function of Bond number is shown in figure 4.16(a). It is clear that as the Bond number increases, the maximum dimensionless volume decreases. As an aside, it is important to note that the drop volume falling off
Figure 4.16: The change of (a) the maximum dimensionless drop volume, and (b) the apparent contact angle of a pendant drop with Bond number.
is always less than the critical volume, first because experimental vibration promotes instability at an earlier stage of drop formation, and second because a small part of the drop remains attached to the needle/holder.

The change of the apparent contact angle of the liquid at the holder with Bond number, at the maximum dimensionless volume, is shown in figure 4.16(b). This figure indicates that for small Bond number values (i.e., a small holder and/or a liquid with a large surface tension value), the apparent contact angle is $90^\circ$ and the liquid is held just above the narrowest point of the neck of the profile. As the Bond number becomes larger (i.e., a larger holder and/or a liquid with a smaller surface tension value), the contact angle at which critical conditions are reached decreases to zero at a Bond number of 10.338. The zero contact angle is the limit for any pendant drop experiment. Further increase in Bond number (i.e., a larger holder and/or a liquid with a smaller surface tension value) results in the shape of the critically stable profile taking a configuration where the holder is not completely wetted. This is the form of a drop as it breaks away from a large orifice such as that of a bath tap. This case is outside the scope of this study.

To further understand the different shapes of critically stable profiles, figure 4.17 shows the pendant drop shapes with the maximum dimensionless volume at different Bond number (0.01, 0.1, 1, and 10). For very small Bond numbers, the apparent contact angle is around $90^\circ$ and the maximum dimensionless volume is very large as expected from figure 4.16. However, the drop shape is very close to spherical. Therefore, it is expected that ADSA will not work satisfactorily in this range. As the Bond number increases, the maximum dimensionless volume and the apparent contact angle decrease as expected from figure 4.16 and the shape of the drop becomes more deformed. Nevertheless, at the high end of the Bond number range, the apparent contact angle approaches zero and the drop shape becomes close to spherical again. Thus, it is expected that ADSA will also not work satisfactorily in this range.
Figure 4.17: The shape of a pendant drop with the maximum dimensionless drop volume for different Bond numbers. The symbols indicate the drop profile, and the lines indicate the best circle that fit the profile.
The shape parameter can be calculated using equation 4.14 for the pendant drop shapes with the maximum dimensionless volume at different Bond numbers. The shape parameter for the largest stable pendant drop as a function of Bond number is shown in figure 4.18. The curve ends at Bond number of 10.338 which is equivalent to zero contact angle. It is clear that the shape parameter is small for very small values as well as large values of Bond number for reasons discussed above. This shows that for the pendant drop case very small values as well as large values of Bond number are not recommended for accurate surface tension measurements.

At this point, it is clear that at any Bond number, there is a critical shape parameter above which the drop shape is considered well deformed. To further investigate this point, experiments were performed to illustrate the change of the critical shape parameter with the Bond number as illustrated below.
4.3.1 Experimental Details

4.3.1.1 Materials and Methodology

For the pendant drop experiments decamethylcyclopentasiloxane (DMCPS) was used rather than OMCTS, mainly due to availability. DEP and water were used again, and a fourth liquid (hexadecane) was added to provide more experiments at a different Bond number. DMCPS $[\text{C}_{10}\text{H}_{30}\text{O}_{5}\text{Si}_{5}]$ was purchased from Sigma-Aldrich Co. (cat. 444278) with purity 97.0% and hexadecane $[\text{C}_{16}\text{H}_{34}]$ from Sigma-Aldrich Co. (cat. 296317) with purity $\geq 99.0\%$. These four liquids have reasonably low vapour pressure and cover a broad range of surface tension. DMCPS, hexadecane, DEP, and water have surface tensions of approximately 18, 27, 36, and 72 mJ/m$^2$, respectively.

Figure 4.19 shows a schematic diagram of the experimental setup for ADSA–PD. A flat circular holder is used to suspend the pendant drop. Except for the pendant drop chamber, the setup is exactly the same as that used for the constrained sessile drop study in Section 4.2.4.1. Three holder sizes were used in this study with outer diameters of 3.08, 4.24 and 6.25 mm. Two holders were used for all four liquids. The smallest holder was used only with one liquid, DMCPS. It is noted that holder sizes different from those used in the constrained sessile drop study were used here. This is mainly due to availability of the holders. Although the same holder radius can be used for both pendant and constrained sessile drop setups, the holder length requirement is different for every setup. Hence, different sets of holders were used in the two types of experiments.
4.3.1.2 Experimental Procedure

All experiments were performed at room temperature of 24°C. It is important to note that the temperature was controlled within one degree. More accurate temperature control requires more sophisticated equipment. Since it is not the purpose of this study to produce literature values of surface tension, such tight control of temperature is not needed here. The experimental procedure used here was exactly the same as in the constrained sessile drop case as shown in Section 4.2.4.2.

In total 9 different experiments were performed to cover a wide range of Bond numbers. Every experiment was repeated at least three times (three runs) to confirm the reproducibility; thus, a total of 27 runs were performed in this study. Each run lasted approximately 200 seconds, implying that approximately 400 images were acquired in each run. Hence the study is relying on approximately 10,000 individual experimental surface tension measurements.

4.3.2 Results and Discussion

The results obtained here are qualitatively similar to the ones obtained for the constrained sessile drop study. Figure 4.20 shows a typical result of ADSA for a pendant drop of hexadecane formed at the end of a holder with outer diameter of 6.25 mm. The figure shows a non-random pattern, and hence lack of consistency with the expectation of a constant surface tension value. The surface tension values of large drops (at the beginning and at the end of every run) are constant, i.e. they do not change with drop size, and they agree with the literature value. However, values calculated from small drops are not constant and show irregular trends, i.e. not just random errors, as shown in figure 4.20. All acquired images were analyzed and the the shape parameter values were calculated according to equation 4.14. To calculate the critical shape parameter for every experiment, the procedure proposed previously [64] was followed. Briefly, the shape...
Figure 4.20: The change of surface tension, drop surface area, drop volume, and the curvature at drop apex with time of a pendant drop of hexadecane formed using a holder with outer diameter of 6.25 mm.
Figure 4.21: The error in surface tension measured by ADSA as a function of the shape parameter for different drop sizes of a pendant drop of hexadecane formed using a holder with outer diameter of 6.25 mm. For a maximum error of $\pm 0.1$ mJ/m$^2$, the critical shape parameter is 0.04.

Parameter values of every run are plotted against the calculated surface tension for the run. The critical shape parameter is the value below which the error in the calculated surface tension value exceeds a specific limit, usually set at $\pm 0.1$ mJ/m$^2$.

The error in surface tension measured as a function of the shape parameter is shown in figure 4.21 for different drop sizes of hexadecane formed with a holder with outer diameter of 6.25 mm. This case is equivalent to a Bond number of 2.72. For a maximum error of $\pm 0.1$ mJ/m$^2$, the critical shape parameter is 0.04. It is important to note that this is a conservative value as seen from the figure. It is to be noted that the measured surface tension is accurate and consistent with an accuracy less than $\pm 0.01$ mJ/m$^2$ for well deformed drops in this run, i.e. for large drop sizes with large shape parameter values. It is also important to note that the overall error in surface tension for this case did not exceed $\pm 0.2$ mJ/m$^2$ for almost all drops in this run, except for very small values of shape parameter below 0.01 where the drop is very small and is essentially a spherical
As in the constrained sessile drop case, it was noted in figure 4.21 that there is a specific trend for the direction (positive or negative) of the error in the surface tension measurements in the pendant drop case. Such trend is repeated when decreasing or increasing the drop volume. Similar trends were also noted in other cases. Figure 4.22(a) shows the error in surface tension of hexadecane formed on a holder with outer diameter of 4.24 mm, while figure 4.22(b) shows the error in surface tension of DEP formed on a holder with outer diameter of 4.24 mm. Both figures show similar – but not exactly the same – trends to that of figure 4.9. Generally, the errors in the pendant drop cases are much smaller than in the constrained sessile drop cases shown in Section 4.2.

The same analysis was applied to experiments using the other three liquids, decamethylcyclopentasiloxane (DMCPS), diethyl phthalate (DEP) and water, using different holders. Following the same procedure shown above, a critical shape parameter value was calculated from every run. All the results are summarized in Table 4.2. The first two columns of the table list the holder size and the liquid used for every experiment. The third column lists the presumably correct surface tension values as found in the literature and/or previous work [217][219]. The fourth column lists the Bond number for every experiment; a range of Bond numbers between 0.61 and 5.02 is covered in this study. The following three columns contain the calculated surface tension value (from the largest drop sizes) and the accuracy of the calculation; more than 60 drops were used in the calculation of the average value and the standard deviation in each run. The last three columns contain the measured critical shape parameter for every experimental run. These values were measured in a procedure similar to that used in figure 4.21.

Inspection of table 4.2 shows that for all systems, except the two listed first and last, respectively, there is excellent agreement from run to run, as well as with the standard values of column 3. It is also noted that the differences from run to run could be due to ambient temperature changes of the order of 0.1°C. The consistency from run to run...
Figure 4.22: The error in surface tension measured by ADSA as a function of the shape parameter for different drop sizes of a pendant drop of: (a) hexadecane formed using a holder with outer diameter of 4.24 mm, and (b) DEP formed using a holder with outer diameter of 4.24 mm. For a maximum error of ±0.1 mJ/m², the critical shape parameters are 0.052 and 0.039, respectively.
Table 4.2: Summary of results of pendant drop experiments using different liquids and different holder sizes.

<table>
<thead>
<tr>
<th>Holder (mm)</th>
<th>Liquid</th>
<th>(\gamma) (mJ/m(^2))</th>
<th>(Bo)</th>
<th>(\gamma_{\text{measured}}) (mJ/m(^2))</th>
<th>(P_{\text{crit}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.24</td>
<td>Water</td>
<td>72.39</td>
<td>0.61</td>
<td>72.033 ± 0.037</td>
<td>72.016 ± 0.026</td>
</tr>
<tr>
<td>3.08</td>
<td>DMCPS</td>
<td>18.26</td>
<td>1.22</td>
<td>18.282 ± 0.004</td>
<td>18.287 ± 0.004</td>
</tr>
<tr>
<td>4.24</td>
<td>HEX</td>
<td>27.17</td>
<td>1.25</td>
<td>27.173 ± 0.011</td>
<td>27.167 ± 0.012</td>
</tr>
<tr>
<td>6.25</td>
<td>Water</td>
<td>72.39</td>
<td>1.32</td>
<td>72.399 ± 0.013</td>
<td>72.411 ± 0.010</td>
</tr>
<tr>
<td>4.24</td>
<td>DEP</td>
<td>36.95</td>
<td>1.33</td>
<td>36.962 ± 0.013</td>
<td>36.935 ± 0.005</td>
</tr>
<tr>
<td>4.24</td>
<td>DMCPS</td>
<td>18.26</td>
<td>2.31</td>
<td>18.360 ± 0.005</td>
<td>18.302 ± 0.005</td>
</tr>
<tr>
<td>6.25</td>
<td>HEX</td>
<td>27.17</td>
<td>2.72</td>
<td>27.194 ± 0.006</td>
<td>27.202 ± 0.004</td>
</tr>
<tr>
<td>6.25</td>
<td>DEP</td>
<td>36.95</td>
<td>2.90</td>
<td>37.035 ± 0.010</td>
<td>37.046 ± 0.008</td>
</tr>
<tr>
<td>6.25</td>
<td>DMCPS</td>
<td>18.26</td>
<td>5.02</td>
<td>17.879 ± 0.002</td>
<td>18.060 ± 0.045</td>
</tr>
</tbody>
</table>
and the accuracy of each run are generally less for the first and last systems, and the agreement with the standard values is much poorer, out of the stipulated error range of ±0.1 mJ/m². A critical shape parameter does not exist for these cases. The reason of this difference in pattern is discussed in detail below.

The change of the critical shape parameter as a function of the Bond number is shown in figure 4.23 for all the experiments of this study. In other words, the critical shape parameter values for every run obtained in a similar fashion to figure 4.21 and summarized in the last three columns of table 4.2 are used to calculate an average critical shape parameter value for every Bond number as shown in figure 4.23. As expected, figure 4.23 shows that the critical shape parameter depends only on Bond number. The critical shape parameter is equivalent to a critical dimensionless volume (the drop volume normalized by the cube of the holder’s radius) that the drop has to exceed to allow for accurate measurement of the surface tension. This can also be understood from figure 4.15 which defines the relationship between the shape parameter, dimensionless volume
and Bond number. The critical shape parameter is one point on every curve below which the drop shape is indistinguishable from the zero gravity shape (circle in this case).

Similar to the constrained sessile drop case, for Bond numbers below one, the largest pendant drop that can be formed with a given holder size is not big enough to calculate an accurate surface tension value. Therefore the critical shape parameters at these conditions, i.e. when the diameter of holder becomes small and approaches zero, cannot be established. Contrary to the constrained sessile drop case, this is also the case for Bond numbers above five, i.e. when the diameter of the holder becomes too large or surface tension becomes too small. This is due to the fact that the largest pendant drop volume that can be formed is not big enough to produce sufficiently non-spherical drops at high Bond number in the pendant drop case. This is in agreement with what was expected from figures 4.16 to 4.18.

The relationship between the critical shape parameter and the Bond number can be used in the design of a pendant drop experiment. Through the above analysis, we know that either very low or very high Bond numbers are not suitable and will not allow accurate surface tension measurement. However, for the constrained sessile drop case, we know that only very low Bond numbers are not suitable. A low Bond number is equivalent to a large surface tension value and/or a small holder size, while a high Bond number is equivalent to a small surface tension value and/or a large holder size. At low Bond numbers (a liquid with large surface tension value used with a small holder size), the pendant drop shape is very close to a sphere (low shape parameter). At high Bond number (a liquid with small surface tension value used with a large holder size), the maximum pendant drop volume is very small and the shape becomes close to a sphere again (low shape parameter). If a rough approximation of the surface tension is known, a suitable holder size can be easily selected to produce accurate measurement. In other words, designing an ADSA–PD experiment to measure a specific surface tension value would require a holder diameter between a minimum and a maximum value to ensure a
Chapter 4. Accuracy and Shape Parameter

maximum error below a specified error tolerance. This would also require that the volume of the pendant drop be large enough so that the calculated shape parameter exceeds the appropriate critical shape parameter as shown in figure 4.23.

For example, based on the calculations presented here and assuming a density difference of 1000 kg/m$^3$ and gravity of 9.80 m/s$^2$, holder diameters between 2.5 and 5.5 mm are recommended for a surface tension value of 15 mJ/m$^2$ to ensure a maximum error of ±0.1 mJ/m$^2$ for large drop sizes. Holder diameters between 4.0 and 8.5 mm are recommended for a surface tension value of 38 mJ/m$^2$. Holder diameters between 5.5 and 12.0 mm are recommended for a surface tension value of 72 mJ/m$^2$. Generally, it is advised to make the drop as large as possible so that the calculated shape parameter exceeds the appropriate critical shape parameter to guarantee accurate results. These rough examples serve as a guide for selecting the right holder size to produce accurate measurement, if a rough approximation of the surface tension is known.

It can be concluded that the use of a usually thin needle or capillary for pendant drop experiments is generally not an optimal choice. No single holder is universally useable. However, a holder size of 5 to 6 mm diameter would be a good choice for most common liquids, if ultimate accuracy is not required. But if ultimate accuracy is required, the choice of the support for a pendant drop experiment for surface tension measurements is extremely important. The size of a suitable holder depends critically on the Bond number, specifically the density difference across the drop interface and the surface tension. Neither holders that are too small or too large are appropriate. It can be seen from table 4.2 that an accuracy near ±0.01 mJ/m$^2$ can be achieved readily.

4.4 Universality of Results

It is believed that the close-to-spherical limitation does exist for ADSA (independent of the details of the numerical modules) as well as other drop shape techniques. To further
investigate this, it is important to test the same results using different modules inside ADSA and other drop shape techniques as well.

The structure of ADSA consists of three main modules. The first module generates the theoretical profiles by numerical integration of the Laplace equation (Axisymmetric Liquid Fluid Interface) “ALFI”. The fifth- and sixth-order Runge-Kutta-Verner pair is used here for the numerical integration [9]. The second module is the image processing, which extracts the experimental profiles of drops. Here, the Canny edge detector is used for the image processing followed by image distortion correction (using a grid) and image enhancement to sub-pixel resolution [8, 220]. The third module is the optimization procedure to find the best fit of the theoretical Laplacian curves to the experimental profile. The Levenberg-Marquardt method is used here for the numerical optimization [7, 9]. In addition to these three modules, there has been one guiding principle: the input must be the simplest conceptually possible, e.g. there must be no need for identifying special points on the drop profile, such as the apex.

The three modules are freely exchangeable against other modules designed for the same purpose. Such a study with different modules was recently performed [218, 219], for a different drop constellation, a sessile drop with a capillary protruding into the drop, ADSA – No Apex (ADSA–NA). To ascertain the general validity of the results in table 4.2, a similar study was performed here for the pendant drop constellation.

The first step is to test the effect of a different image processing technique. The Canny edge detector was replaced by a robust non-gradient edge detector called SUSAN [221]. SUSAN is a non-gradient edge detection operator; it does not depend on intensity gradients in the digitized image. This was followed by the standard ADSA procedure. The results of this step “ADSA_{SUS}” for the case of a pendant drop of hexadecane formed using a holder with outer diameter of 6.25 mm are shown in figures 4.24(b) and 4.25. The computation time was less than one second per image on a computer with a 2.66 GHz CPU and 3.00 GB of RAM. This is similar to that of the standard ADSA.
Next, the effect of a different numerical optimization technique is considered. The Levenberg-Marquardt method was replaced by the non-gradient Nelder-Mead simplex method [222]. Compared to the gradient methods, Nelder-Mead is computationally easier to implement since it does not require a derivative of the objective function, but it consumes more computer time. The results of this step “ADSA_{NM}” for the case of the same pendant drop of hexadecane formed with the same holder are shown in figures 4.24(c) and 4.25. The computation time was roughly 3 to 4 seconds per image.

The third step is to test a different drop shape technique called theoretical image fitting analysis “TIFA” [211, 223] that uses a completely different approach from ADSA. The difference between the strategy of ADSA and TIFA is that, in TIFA, the interfacial properties are calculated by fitting the whole theoretical image – not the drop profile only – to the experimental image of the drop. The theoretical image is a black and white image of the drop generated by numerically solving the Laplace equation. An error function measures the pixel-by-pixel difference between the gradient of the whole theoretical and experimental images. The interfacial properties are found by fitting the gradient of the theoretical image to the gradient of the experimental one by minimizing the error function. The remarkable feature of TIFA is that it operates without using edge detection algorithms. In fact, image analysis is tied to the optimization process in TIFA, and it is not a separate module as in ADSA. The results of this step, using TIFA for the case of the same pendant drop of hexadecane formed with the same holder are also shown in figures 4.24(d) and 4.25. The computation time was approximately 72 seconds per image (approximately 8 hours for this run).

Figure 4.24 shows the comparison between the surface tension values of a pendant drop of hexadecane formed using a holder with outer diameter of 6.25 mm calculated using the standard ADSA algorithm, the modified algorithms (ADSA_{SUS} and ADSA_{NM}), and the TIFA algorithm. The calculated surface tension values for the large drops are very consistent for all four algorithms, with values of $27.202\pm0.004\ \text{mJ/m}^2$, $27.208\pm0.004\ \text{mJ/m}^2$.
Figure 4.24: The change of surface tension with time of a pendant drop of hexadecane formed using a holder with outer diameter of 6.25 mm. The calculations are repeated using different software options; ADSA: the default options using Canny for edge detection and Levenberg-Marquardt for optimization; ADSA<sub>SUS</sub>: SUSAN is used instead of Canny; ADSA<sub>NM</sub>: Nelder-Mead is used instead of Levenberg-Marquardt; TIFA: theoretical image fitting analysis.
Figure 4.25: The error in measured surface tension as a function of the shape parameter for different drop sizes of a pendant drop of hexadecane formed using a holder with outer diameter of 6.25 mm. The calculations are repeated using different software options; ADSA: the default options using Canny for edge detection and Levenberg-Marquardt for optimization; ADSA\textsubscript{SUS}: SUSAN is used instead of Canny; ADSA\textsubscript{NM}: Nelder-Mead is used instead of Levenberg-Marquardt; TIFA: theoretical image fitting analysis.

mJ/m\(^2\), 27.201±0.004 mJ/m\(^2\), and 27.194±0.005 mJ/m\(^2\), respectively. For the small drops, all algorithms fail to predict the correct value for the surface tension. Interestingly, the ADSA algorithms are very consistent from one ADSA version to the next, even for small drops. However, the TIFA algorithms show larger deviation from the correct surface tension value in the case of small drops.

The error in surface tension measured by these four different algorithms as a function of the shape parameter is shown in figure 4.25 for the same run. The original and modified ADSA algorithms are very consistent and show very similar trends. It is important to note that the overall error in surface tension for this case did not exceed ±0.2 mJ/m\(^2\) for almost all drops in this run, except for very small values of shape parameter below 0.01 where the drop is very small and is essentially a spherical cap. The TIFA algorithm shows
similar results for large drops (large shape parameter) but deviates dramatically for small drops (small shape parameter). For a maximum error of $\pm 0.1 \text{ mJ/m}^2$, a conservative critical shape parameter value is 0.04 in this case.

Stepping well back, it is apparent that, for drops of a reasonable size, high quality surface tension results are readily obtainable from drop shape techniques. While the choice of optimization method has a considerable effect on computation time and necessary accuracy of the initial guess, it does not have an appreciable effect on the surface tension result, see specifically figures 4.24(a) and 4.24(c). Less expected is the finding that edge detection also does not have a significant effect; the standard ADSA using Levenberg-Marquardt and Canny (figure 4.24(a)) or alternatively Levenberg-Marquardt and SUSAN, i.e. a non-gradient edge detection strategy (figure 4.24(b)), agree well. A method totally outside the ADSA scheme, TIFA, also yields the same results, for large enough drops.

Finally, the results show that all techniques are outside the stipulated error range of $\pm 0.1 \text{ mJ/m}^2$ when the drop or bubble approaches spherical shapes. Using non-gradient edge detection or non-gradient optimization modules did not change the results. It was noted that TIFA techniques work well for well deformed drops but yield erroneous results (worse than ADSA) for close-to-spherical drops. This is in agreement with other studies in the literature [219].
Chapter 5

Summary of Progress and Outlook

The ADSA–CSD configuration has been further developed to produce a highly versatile methodology for surface tension measurements. ADSA–CSD was used to probe the behaviour of films adsorbed or spread at air-water interfaces. During these studies it became clear that the accuracy of the data needed to be quantified and measured. In Chapter 4, a derivation of a versatile assessment of the accuracy of surface tension measurement is described. This represents a fundamental improvement which can be applied to other shape dependant configurations.

The accuracy of any drop shape technique will depend on the deviation of an experimental drop shape from the zero gravity shape, i.e. the spherical shape in the case of sessile/pendant drops considered here. This difference between the two shapes can be expressed quantitatively as a shape parameter. By comparing shape parameters with the deviations of calculated surface tensions for different drop sizes of liquids of known surface tension, a critical shape parameter was established, below which surface tension measurements will exceed a specified error tolerance. The key element of this procedure is a dimensional analysis used to describe similarity in constrained sessile drop and pendant drop shapes. The shape parameter concept was used in Chapter 4 to quantify the accuracy of both the constrained sessile drop (ADSA–CSD) and the pendant drop
(ADSA–PD) constellations. It was shown that an accuracy near ±0.01 mJ/m² can be achieved readily for well deformed drops.

It is known that the pendant drop constellation yields the most accurate results among the drop shape methods. However, in the case of low surface tension values, gravity tends to detach the drop from the capillary holder before it is large enough [71], making surface tension measurement impossible. For such low surface tensions, the constrained sessile drop constellation is more suitable. Such low surface tension values (near 1 mJ/m²) are very common in lung surfactant studies. The analysis presented in Chapter 4 can be used in the design of constrained sessile drop and pendant drop experiments for surface tension measurement. If a rough approximation of the surface tension is known, a holder of suitable size for a specified accuracy can be easily selected. The size of a suitable holder depends critically on the Bond number, which in turn depends on the density difference across the drop interface and the surface tension.

It was shown in Chapter 4 that the use of different edge detectors or optimization techniques is not expected to improve the accuracy of the surface tension measurements. If further progress on accuracy is needed, it is recommended to look further into the quality of the images obtained. Specifically the optics of the whole system, lighting and refraction, and possibly image magnification are to be considered. It is doubtful if accuracy can be enhanced much but possibly the range of critical shape parameter and the working range of Bond numbers for different constellations might be improved (cf. tables 4.1 and 4.2). A suggested strategy would be to repeat the pure liquids experiments and shape parameter analysis done in Chapter 4 with different optics. Any such work would need extreme temperature control.

Using the high accuracy now obtained, the pendant drop method can be used for many applications. Thus, more accurate temperature dependant surface tension measurement data could be produced. For example, as temperature coefficients of surface tension are generally in the order of 0.1 mJ/m²·K, using a method with accuracy of ±0.1
mJ/m² requires measurements at a much wider temperature range than is needed in a method with accuracy of ±0.01 mJ/m². The pendant drop technique has the potential of being used to determine the effect of temperature on surface tension with very high accuracy. Most of the present literature data for temperature dependant surface tension measurement were produced a long time ago with methods such as the capillary rise, the maximum bubble pressure, or the drop weight [224]. These methods have limitations of which ADSA–PD is free [93, 225]. The capillary rise method requires a very sophisticated setup, the maximum bubble pressure method assumes a spherical bubble, and the drop weight method relies heavily on an empirically determined correction factor [93, 225]. Therefore, there is considerable potential in drop shape techniques due to the compactness and complete isolation of the experimental setup, allowing for various experimental conditions ranging from high pressure and high temperature to high and ultra-high vacuum. The only problem was uncertainty about accuracy which was removed in Chapter 4.

The shape parameter analysis presented here is also applicable – in principle – to other configurations such as unconstrained sessile drops, captive bubbles, liquid bridges, and liquid lenses. However, the comparison with a circle will only apply to sessile/pendant drops and bubbles. For other configurations, the drop shape must be compared to the respective zero gravity shape for each case. These drops at zero gravity may assume different shapes including cylindrical, nodoidal, unduloidal and catenoidal. The radius of the drop holder can still be used for normalization in the case of a liquid bridge. It can be replaced by the radius of the capillary in the case of pendant drop or the contact radius in the cases of unconstrained sessile drop or liquid lens. For the liquid bridge case, the radius of the upper holder will be a new parameter that has to be considered in defining the shape. For the unconstrained sessile drop and liquid lens cases, the diameter of the pedestal (holder) does not exist. The contact angle and contact radius will play an important role in defining the shape of the drop for these cases.
In the lung surfactant context, the performance of surfactant preparations were assessed using the dynamic surface tension response to film compression and expansion. Prior to this work, typically only the minimum surface tension values at the end of compression were reported. Several distinctive physical surface effects are expected to play a role in the process of film compression and expansion: elasticity, adsorption and desorption. So far only the elasticity during compression had been considered by determining the slope of the surface tension versus surface area plot. There are two concerns with this procedure. First, as seen in Chapter 4, the accuracy of the surface tension and indeed the surface tension value itself determined by ADSA can depend on drop size, unless all drop sizes are associated with a sufficiently large shape parameter. If such a trend of erroneous surface tension occurs, the error in the elasticity as a slope of surface tension versus area can be quite large. The results of Chapter 4 show how to avoid these errors. Second, relying on the slope of the surface tension response to the surface area changes as a strategy to determine elasticity assumes that there is no simultaneous change of surface concentration, say during compression. This assumption is at variance with known experimental facts of squeeze out, relaxation and spreading. This difficulty is removed by the model described in Chapter 3 by allowing the surface tension to change due to simultaneous effects of one or more of these dynamic phenomena. A dynamic compression-relaxation model (CRM) was developed and used to describe the mechanical dynamic properties (elasticity, relaxation and adsorption) of insoluble pulmonary surfactant films by investigating the response of surface tension to changes in surface area. Multi-variable optimization was introduced here to calculate the dynamic parameters from experimental results of dynamic surface tension measurements. A detailed sensitivity study for CRM was also performed in Chapter 3.

Combined with the unique capabilities of ADSA–CSD, CRM was used to study the effect of humidity, concentration, compression ratio and frequency of a lung surfactant preparation, BLES. The highest concentration used was 27 mg/ml, similar to that used
clinically. Low surface tension at such high concentrations are very difficult to measure using other methods as discussed in Chapter 1. While the dynamic properties of BLES-only preparations do not improve significantly with increasing BLES concentration, the same might not be true in the presence of inhibitors. Using ADSA–CSD, CRM studies of the dynamic properties of lung surfactant preparations can be extended to more complex systems containing inhibitors.

There is an urgent need to be able to access the air/liquid interface from both sides. This need arises from clinical reality: contaminations (pollution) affect the surfactant interface from the air side and therapeutical surfactant preparations are instilled to the lungs from the air side, while leakage of plasma and blood proteins can occur from the liquid side causing severe lung injury. Therefore, accessing the lung surfactant interface from both sides is a desirable feature in any in vitro technique. The ADSA–CSD setup was further developed in Chapter 2 to allow for such studies.

Two main capabilities were added to the ADSA–CSD setup. The first one was the deposition capability which allows spreading of material onto the air/liquid interface from the air side. This permits the study of the collapse of deposited films through the study of pure monolayers as well as mixed monolayers. DPPC and DPPG monolayers, which are the main components of most commercial lung surfactant preparations (Appendix A), were studied to characterize the role of such molecules in maintaining stable film properties and surface activity of lung surfactant preparations. The second capability added was the double injection which facilitates the complete exchange of the subphase of a spread or adsorbed film from the liquid side. The development was illustrated through inhibition studies using serum, albumin, fibrinogen, and cholesterol. Evaluation of lung surfactant inhibition, resistance and reversal under completely controlled physiological conditions is illustrated in Chapter 2. It is noted that the redesigned ADSA–CSD is a versatile tool for surface tension measurements that has a wide range of applications outside the scope of the lung surfactant research area as well.
Chapter 5. Summary of Progress and Outlook

Clinical lung surfactant therapies currently use mechanical ventilation, and more recently High Frequency (more than 1 Hz) Ventilation (HFV). However, little is known about the role of surfactants in high frequency ventilation. The present ADSA–CSD setup is not capable of simulating the high frequency cycling of more than 1 Hz due to mechanical limitations of the servo-mechanism controlling the syringe. However, high frequency studies can be readily facilitated in ADSA–CSD by straightforward hardware upgrades. Two main items are needed: a piezo-actuator with a dedicated computer-programmable controller to facilitate the low amplitude high frequency oscillations of the drop volume, and a high speed camera. Both are readily available commercially.

The future high frequency ADSA–CSD can be used in conjunction with CRM to characterize the surface activity of relevant lung surfactant preparations at higher frequency, and to study the changes of the dynamic properties (elasticity, relaxation, and adsorption) at high rates of film compression and expansion. A high frequency ADSA–CSD methodology and the ability to characterize dynamic interfacial properties using CRM at high frequency will have great potential for a wide range of colloid and interfacial applications, beyond lung surfactants.
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Note: All references starting with Ref. 226 are only used in Appendix A.
Appendix A

Commercial Lung Surfactants*

Several classification systems are used for commercial exogenous lung extracts [1 174 175 226]. Figure A.1 divide different commercial lung extracts into two main categories according to the source of the preparation. The first category is natural lung surfactants which contains endogenous lung extracts from animals. The second category is synthetic lung surfactants which does not contain any natural extracts. Generally, preclinical animal testing and clinical trials suggest that the performance of natural animal-derived preparations is much better than synthetic preparations [227 229]. However, batch to batch variations, low content of surfactant proteins and high cost are among the limitations of natural animal-derived preparations [230 231].

A.1 Natural Surfactant Extracts

Natural surfactants [230 234] can also be classified into three categories. The first contains exogenous surfactants extracted by animal lung lavage (Alveofact, BLES, Infasurf). The general extraction procedure is summarized in figure A.2. The second group contains exogenous surfactants processed from animal lung tissue (Curosurf). The third group is

*References used here are contained in the bibliography list at the end of the body of the thesis.
Figure A.1: General classification of commercial lung extracts.
similar to the second one; however, these surfactants are supplemented with synthetic additives (Surfacten, Survanta). The general extraction procedure of second and third categories is summarized in figure A.3. A comparison of the composition of the six natural surfactant extracts is shown in figure A.4. More details about natural surfactants can be found in a recent review [235].

A.1.1 Alveofact

Alveofact (SF-RI 1 or bovactant) is a clinical exogenous lung surfactant obtained from bronchoalveolar lavage of bovine lung surfactant [236–240]. The lavage is pelleted by centrifugation, followed by extraction with chloroform methanol to remove hydrophillic surfactant protein (see figure A.2). It contains weight distribution of 99% phospholipids
Figure A.3: Extraction procedure for surfactants extract of lung tissue.
Figure A.4: Composition of natural surfactants extracts.
Appendix A. Commercial Lung Surfactants

(PL) and neutral lipids (NL) [that include 4% cholesterol], and 1% hydrophobic surfactant proteins SP-B and SP-C as shown in figure A.4(a). It is formed clinically as a sterile suspension in 0.15 M NaCl at 45 mg/ml with dosing amount of 1.2 ml/kg. Alveofact is produced by Thomae GmbH, Biberach, Germany; and Boehringer Ingelheim Co., Ingelheim, Germany.

A.1.2 BLES

BLES (bovine lipid extract surfactant) is a clinical exogenous lung surfactant made from of lung surfactant lavaged from cows [241–244]. The lavage is pelleted by centrifugation, followed by extraction with chloroform methanol to give BLSE (bovine lung surfactant extract). Additional extraction with acetone is used to remove cholesterol and other neutral lipids (see figure A.2). It contains weight distribution of 98-99% phospholipids (PL) [that include 79% phophatidyl-choline (PC)], and 1% hydrophobic surfactant proteins SP-B and SP-C as shown in figure A.4(b). It is formed clinically as a sterile suspension in 0.15 M NaCl and 1.5 mM CaCl\(_2\) at 25 mg/ml with dosing amount of 5 ml/kg (135 mg/kg), 1-2 doses every 8 h. BLES is produced by BLES Biochemicals Inc., London, Ontario, Canada.

A.1.3 Infasurf

Infasurf (CLSE or calfactant) is a clinical exogenous lung surfactant obtained from calves lung lavage (CLSE) [245–257]. The lavage is pelleted by centrifugation at 10,000 - 12,000 \(\times\)g, followed by extraction with 2:1 chloroform methanol to give CLSE or calf lung surfactant extract (see figure A.2). It contains weight distribution of 93% phospholipids (PL) [that include 83% phophatidyl-choline (PC), 6% phosphatidyl-glycerol (PG), 4% phosphatidyl-inositol (PI) and phosphatidyl-serine (PS), 3% phosphatidyl-ethanolamine (PE), and 2% sphingomyelin (SPH)], 5% cholesterol and neutral lipids (NL), and 1.5% hydrophobic surfactant proteins SP-B and SP-C as shown in figure A.4(c). Desaturated
Appendix A. Commercial Lung Surfactants

PC is approximately 50% of the total phospholipids. It is formed clinically as a heat-sterilized suspension in 0.15 M NaCl at 35 mg/ml with dosing amount of 3 ml/kg (105 mg/kg), 1-4 doses every 6-12 h. Infasurf is produced by ONY Inc., Amherst, New York, USA (for Forest Pharmaceuticals Inc., St. Louis, Missouri, USA).

A.1.4 Curosurf

Curosurf (poractant alpha) is a clinical exogenous lung surfactant obtained from minced porcine lung tissue \([258-264]\). The process include washing, centrifugation at 1,000 - 3,000 \(\times g\), followed by extraction with 2:1 chloroform methanol, and liquid-gel affinity chromatography (see figure A.3). It contains weight distribution of 93% phospholipids (PL) [that include 67-75% phosphatidyl-choline (PC), 12-22% phosphatidyl-ethanolamine (PE) and sphingomyelin (SPH)], and 1% hydrophobic surfactant proteins SP-B and SP-C as shown in figure A.4(d). It is formed clinically as a suspension sterilized by high pressure filter system (0.45 and 0.2 \(\mu\)m filters), dried or lyophilized and stored in vials then suspended in saline by sonication at 80 mg/ml prior to use with dosing amount of 2.5 ml/kg (200 mg/kg) to 1.25 ml/kg (100 mg/kg). Curosurf is produced by Chiesi Farmaceutici, Parma, Italy.

A.1.5 Surfacten

Surfacten (Surfactant TA) is a clinical exogenous lung surfactant made by organic solvent extraction of finely ground bovine lung tissue supplemented with synthetic DPPC, palmitic acid, and tripalmitin \([265-270]\). The lung tissue goes through several centrifugation and floatation processes, extraction with ethyl acetate to reduce neutral lipids, and additional extraction with chloroform methanol to remove ethyl acetate. The extract is then supplemented with the aforementioned synthetic additives (see figure A.3). The final product contains weight distribution of 84% phospholipids (PL), 8% palmitic acid, 7% tripalmitin, and 1% hydrophobic surfactant proteins SP-B and SP-C as shown in
Appendix A. Commercial Lung Surfactants

figure A.4(e). It is formed clinically as a suspension sterilized by high pressure filter, lyophilized and stored in vials then suspended in sterile 0.15 M NaCl at 30 mg/ml prior to use with dosing amount of 4 ml/kg (100 mg/kg), 1-4 doses every 6 h. Surfacten is produced by Mitsubishi Tanabe Pharma Corporation (formerly Tokyo Tanabe Co. Ltd.), Tokyo, Japan.

A.1.6 Survanta

Survanta (beractant) is a clinical exogenous lung surfactant made by organic solvent extraction of processed and supplemented bovine lung tissue [230, 255, 263, 271–279]. It is prepared by similar methods and identical synthetic additives as in Surfactant TA (see figure A.3). The final product contains hydrophobic surfactant proteins slightly less than 1% (include a small amount of SP-B) as shown in figure A.4(f). It is formed clinically as an autoclave-sterilized suspension in 0.15 M NaCl at 25 mg/ml with dosing amount of 4 ml/kg (100 mg/kg), 1-4 doses every 6 h. Survanta is produced by Abbott Laboratories, Abbott Park, Illinois, USA.

A.2 Synthetic Surfactants

Synthetic Surfactants [280, 282] can also be classified into three categories. The first contains synthetic exogenous surfactants with no proteins (ALEC, Exosurf). The second group contains synthetic exogenous surfactants with synthetic simplified peptides (Surfaxin). The third group contains synthetic exogenous surfactants with recombinant human apoproteins (Venticute). A comparison of the composition of the four synthetic surfactants is shown in figure A.5. More details about synthetic surfactants can be found in recent reviews [280, 283, 288].
Appendix A. Commercial Lung Surfactants

Figure A.5: Composition of synthetic surfactants.
A.2.1 ALEC

ALEC (artificial lung expanding compound) is a clinical exogenous lung surfactant containing synthetic dipalmitoyl-phosphatidyl-choline (DPPC) and egg phosphatidyl-glycerol (PG) in a 7:3 molar ratio \[79-81, 289, 290\] as shown in figure A.5(a). DPPC is the main component for lowering surface tension, while egg PG is to improve adsorption and spreading. It is formed clinically as a fine white powder mixed (below 30°C) with sterile 0.15 M NaCl. It is produced by Britannia Pharmaceuticals Ltd., Redhill, Surry, United Kingdom. ALEC was withdrawn from the market few years ago.

A.2.2 Exosurf

Exosurf (colfosceril palmitate) is a clinical exogenous lung surfactant containing synthetic dipalmitoyl-phosphatidyl-choline (DPPC), hexadecanol, and tyloxapol in a 1:0.111:0.075 weight ratio \[252, 254, 279, 291-300\] as shown in figure A.5(b). DPPC is the main component for lowering surface tension, while the nonionic detergent and the C16:0 alchohol hexadeconal are to improve adsorption and spreading. It is formed clinically as a sterile lyophilized powder in vaccum-sealed vials with 0.1 M NaCl then suspended in 8 ml of distilled water prior to use with dosing amount of 5 ml/kg (67.5 mg/kg), 1-4 doses every 12 h. It is produced by GlaxoSmithKline (formerly Burroughs Wellcome), Research Triangle Park, North Carolina, USA. Exosurf is no longer available in USA and Canada.

A.2.3 Surfaxin

Surfaxin (KL4 or lucinactant) is a clinical exogenous lung surfactant containing synthetic dipalmitoyl-phosphatidyl-choline (DPPC), palmitoyl-oleoyl-phosphatidyl-glycerol (POPG), palmitic acid, and a 21 amino acid synthetic hydrophobic peptide with repeated sequences of one lysine (K) and four leucine (L) residues \[82-84, 301-314\]. DPPC and
POPG are present in 3:1 weight ratio. Palmitic acid is present at 15% by weight relative to phospholipids, while the hydrophobic peptide (known as KL4 or sinapultide) is present at 3% by weight as shown in figure A.5(c). It is produced by Discovery Laboratories Inc., Warrington, Pennsylvania, USA. Recently, an aerosolized version of Surfaxin was developed by the same producer called Aerosurf. Surfaxin and Aerosurf are investigational drug products, and are not approved for use yet.

A.2.4 Venticute

Venticute (Recombinant SP-C or lusupultide) is a clinical exogenous lung surfactant containing synthetic dipalmitoyl-phosphatidyl-choline (DPPC), palmitoyl-oleoyl-phosphatidyl-glycerol (POPG), palmitic acid, and human sequence or modified human sequence recombinant surfactant apoprotein (rSP-C) [85, 184, 198, 315, 320]. DPPC and POPG are present in 7:3 weight ratio. Palmitic acid is present at 5% by weight relative to phospholipids, while the rSP-C protein is present at 2% by weight as shown in figure A.5(d). It is produced by Altana Pharma AG (formerly Byk Gulden), Konstanz, Germany. Venticute is an investigational drug product, and is not approved for use yet.