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0-612-28040-3
Abstract

This thesis applies principles and methodologies of surface thermodynamics to problems of lung physiology. In order for the muscular system to be able to perform the work of expansion and compression of the lung, the surface tension must be as low as possible. Nature facilitates this by providing a lung surfactant, consisting of a mixture of lipids and proteins, which are transported and maintained at the surface of the lung. Correct surface tension measurements of such systems have become available only very recently. These measurements have been cumbersome and based on assumptions, the correctness of which could not be guaranteed generally.

In order to overcome the current difficulties, a methodology, Axisymmetric Drop Shape Analysis-Captive Bubble (ADSA-CB), was developed for surface tension measurement. This new technique allows mimicking of the periodic inflation and deflation of the lung while measuring thousands of surface tension values in a very short time. With this technique, the so-called “squeeze-out” and film collapse phenomena were examined closely.

An important aspect of lung surfactant is the fact that, ultimately, the surface film will be composed almost exclusively of lipids, mostly dipalmitoyl phosphatidyl choline (DPPC). By means of an ADSA study on the DPPC monolayer, a mechanism of lipid arrangement at the surface was proposed: gaseous phase, gradual transition from gaseous to liquid-condensed and to solid phase, and solid phase.

To develop expressions for the key performance measures of lungs, such as surface area, distortion energy and mechanical work, in terms of measurable quantities, a thermodynamic model of the lung has been developed: Starting with a general thermodynamic expression for the distortion energy, a relation between the recoil pressure and the surface tension was found. Using the
properties of partial derivatives, a general expression for the surface area was derived. For the two separate cases: 1) lungs with constant surface tension and 2) air-filled lungs, explicit expressions for the distortion energy and mechanical work were obtained. The model predictions are in good agreement with the experiment in all situations.
Professor D.E. Cormack  
Associate Dean – Division III  
School of Graduate Studies  
University of Toronto  
65 St. George Street  

Dear Professor Cormack:  

SUBJECT: Doctoral Program of Mr. R.M. Prokop  

You may recall that I spoke to you some time ago about the fact that Rob Prokop, one of our Doctoral candidates, died while attending a conference with his Thesis Supervisor, Professor A.W. Neumann, and some of his fellow graduate students. This unfortunate event took place on June 18th, 1996. The purpose of my letter to you is to propose that the degree of Doctor of Philosophy be conferred on Rob Prokop posthumously.

Rob Prokop is a graduate of the University of Alberta, receiving a Bachelor of Science in Mechanical Engineering with Distinction in 1992. He subsequently completed the degree of Master of Science in Mechanical Engineering in 1993 with a GPA of 8.3/9.0. At the University of Toronto, he completed his Doctoral program course requirements receiving three grades of A+ and two grades of A. Clearly, Rob Prokop's academic record was outstanding throughout all three degree programs he pursued.

In the area of research, Rob Prokop was outstanding. Although he was in the early years of his research career, his publication list is truly outstanding. He has 11 refereed journal publications which are published, in press, or in preparation at the time this letter was written. The manuscripts which were not completed at the time of Rob's passing are being completed by his co-authors. As impressive as the quantity of his publications are, the quality and the significance of Rob's research are equally impressive. Ongoing research, based on the work started by Rob Prokop, will have great significance in the area of lung modelling, understanding the processes which take place in the lung, and the treatment of lung disorders.

Enclosed are documents which will support the statements made above, and expand on them. These documents are:

1. Letters from Academic Staff Members
   (a) Professor A.W. Neumann  
   (b) Professor R.D. Venter  
   (c) Professor B. Benhabib  
   (d) Professor C.R. Ethier  

   Thesis Supervisor  
   Vice-Dean,  
   Faculty of Applied Science and Engineering  
   Associate Chair (Graduate Studies)  
   Member of Rob Prokop's Ph.D. Qualifying Exam Committee
2. Statements from Colleagues
   (a) Mr. Ajay Ivoti
   (b) Mr. Pu Chen

3. Curriculum Vitae of Rob Prokop

4. List of Publications by Rob Prokop
   (a) List of Ph.D. Related Publications
   (b) Reprints, Copies of Publications

In summary, Rob Prokop was an outstanding doctoral candidate who was well on his way to completing the requirements of an outstanding thesis. He is the type of person that we would have been proud to have as a member of the academic staff here. His untimely death on June 15, 1996, deprived Rob of these achievements and us of the benefits of his achievements. In recognition of the significant contributions made by Rob Prokop while at the University of Toronto. I strongly recommend that the degree of Doctor of Philosophy be conferred on him posthumously.

Yours sincerely,

\[signature\]

I.G. Currie
Professor and Chair
I. G. Currie
Professor and Chair
Department of Mechanical and
Industrial Engineering
University of Toronto
5 King's College Road
Toronto, Ontario
M5S 3G8

Dear Professor Currie:

SUBJECT: Mr. Rob M. Prokop – Ph.D. Posthumously

I write to you to request that you initiate procedures to confer a Ph.D. degree on Rob Prokop posthumously. As you know, Rob died suddenly in June 1996, while attending a conference in the U.S.A. For your information, I provide, below, background and relevant information.

Rob Prokop’s task for his Ph.D. studies was to apply principles and methodologies of surface thermodynamics and of engineering sciences in general to problems of lung physiology. In the respiratory process, lung volume and lung surface area change periodically, accompanied by changes in the bulk pressure and the surface tension of the surface of the lung. The latter point is of immense importance: In order for the muscular system to be able to perform the work of expansion and compression, the surface tension must be as low as possible. Nature facilitates this by providing a lung surfactant, consisting of a mixture of lipids and proteins, which are transported and maintained at the surface of the lung. Correct surface tension measurements of such systems have become available only very recently. These measurements have been cumbersome and based on assumptions, the correctness of which could not be guaranteed generally.

The key issue for Rob Prokop was therefore the development of a methodology for surface tension measurement which allowed mimicking the periodic inflation and deflation of the lung, and which had to be highly automated to allow for thousands of measurements in a very short time. The solution of the problem is described in publication No. 6 (accepted). An account of the underlying applied mathematics was published earlier (Publication No. 1), and physiologically important aspects of the surface tension measurements will be reported in publication No. 9. The methodology developed is very general, and applicable to systems far away from lung surfactant. Publication No. 2 describes a study of a block-copolymer surfactant at a liquid/liquid interface, by means of the same methodology.

The work on lung surfactants initiated in our Laboratory for Applied Surface Thermodynamics by Rob Prokop found quick recognition: We were invited to write a review in Current Opinion in Colloid and Interface Science. Publication No. 3. After only minimum discussion Rob Prokop completed, two days before his death, a draft. As with all of his work, the “draft” required nothing more than a word here or a comma there.

An important aspect of lung surfactant is the very recent insight that, ultimately, the surface film will be composed almost exclusively of lipids, mostly dipalmitoyl phosphatidyl choline (DPPC). The study of the physical chemical properties of such films under dynamic conditions as they prevail in vivo therefore was an
urgent task that could be performed readily with our novel methodology. The task fell to Ajay Jyoti, who, with guidance almost exclusively from Rob Prokop, produced two important papers (Publications No. 4 and 5) within the framework of his M.A.Sc. thesis.

While these two papers explored structural aspects of monolayers of highly purified lipids in a "pure science" mode, Publication No. 7 (draft attached) is at the opposite clinical end of the range of Rob Prokop's efforts. In collaboration with medical personnel from the Hospital for Sick Children, Rob Prokop studied properties of lung surfactant as obtained from lavages of animal lungs.

Publication No. 8 is an impressive piece of theoretical work of Rob Prokop. While he had firm plans for at least three papers on lung modelling, we will have to be content for now with this one important document. The primary purpose of the paper is to develop expressions for the key performance measures of lungs, such as surface area, the distortion energy and mechanical work, in terms of measurable quantities. Rob Prokop started up his procedure by drawing on his profound thermodynamic insight, through an ingenious use of Maxwell relations.

Finally, Publications No. 10 and 11 are book chapters which were planned in considerable detail, with Rob Prokop spear-heading the effort. The chapters will now be completed by Pu Chen, Rob's colleague and close friend.

Since the eleven publications stemming from Rob Prokop's Ph.D. efforts usually have several authors. I would like to discuss both my procedure with respect to authorship as well as the status and contribution of the other authors. With respect to my procedure: The person who was the driving force and was responsible for a study will be the first author. For all work for which I am the primary supervisor and which I control, I am the last author. All other authors appear more or less in the sequence of decreasing input. I should also state that it is relatively easy in my laboratory to become a co-author.

With respect to status and contribution of the other authors:

**Publication No. 1:** N. Niyakan was my M.A.Sc. student. In my graduate course, MEC1109 – Surface Phenomena, I require a term paper preferably presenting original work. N. Niyakan produced a document, under the guidance of a Ph.D. student, Oscar del Rio, which provided a starting point for Rob Prokop's development.

**Publication No. 2:** Dr. M.L. Hair is an Adjunct Professor of our department who realized that the methodologies developed by Rob Prokop would be ideally suited to study di-block co-polymers at liquid/liquid interfaces. Further studies in this direction are now under way following this well-received first study.

**Publication No. 3:** See above.

**Publications No. 4 and 5:** Work performed by Ajay Jyoti in his M.A.Sc. thesis under the guidance of Rob Prokop. D.Y. Kwok is a present Ph.D. Student who had pioneered aspects of the methodology employed by Ajay Jyoti in his M.A.Sc. thesis. D.Y. Kwok pursues his Ph.D. studies in an area not related to this topic. J. Li, D. Vollhardt, R. Miller and H. Möhwald are colleagues at the Max Planck Institut for Colloids and Surfaces in Berlin, who provided independent structural information about the monolayers through Brewster Angle Microscopy (BAM).

**Publication No. 6:** M. Esfahani, A. Garg, M. Mihaila are undergraduate students who worked under the supervision of Rob Prokop. O. del Rio, a Ph.D. student, supplied assistance with applied mathematics. S. Susnar, also a Ph.D. student, assisted with certain design matters. Z. Policova, my long-term technician, assisted with laboratory procedures.
Publication No. 7: Dr. P. Cox is the present and Dr. C. Bryan the emeritus director of intensive respiratory care, the Hospital for Sick Children, and H. Frendova a researcher associated with Dr. Bryan.

Publication No. 8: P. Chen is a Ph.D. student who has kindly agreed to finalize this publication as well as No. 11 and 12.

Publications No. 9 to 11: All authors were discussed above.

Rob Prokop was also a co-author of presentations at national and international conferences. In the list of conference papers, the speaker's name is underlined. Rob Prokop delivered his papers effectively with competence and confidence, which would have done even a much more senior investigator proud.

Summary: We have in these 11 publications, a substantial amount of excellent work by Rob Prokop. While we do not have a Ph.D. thesis, the publication record certainly compares favourably with the publications typically arising from a Ph.D. thesis. I feel very strongly that in terms of quantity and even more so in terms of quality that we have an opus which merits a Ph.D.

Please let me know if I can be of further assistance.

Yours sincerely,

[Signature]

A. W. Neumann
Professor
I.G. Currie  
Professor and Chair  
Department of Mechanical and  
Industrial Engineering  
University of Toronto  
5 King's College Road  
Toronto, Ontario  
M5S 3G8

Dear Professor Currie:

SUBJECT: Mr. Rob M. Prokop

I am pleased to support the nomination of Rob Prokop for a Ph.D., posthumously. I was a member of Rob's Ph.D. qualifying exam committee, and had talked with Rob informally a number of times about his research.

Rob's performance in his Ph.D. exam was amazing. Rob had a rare combination of skills: he had a superb mastery of the technical material at hand, as well as the ability to express and explain that material in a lucid and concise fashion. It was clear to me after only a few questions during the course of his qualifying exam that Rob had "what it takes" to be a successful Ph.D. candidate, and more. In fact, I confess that I enjoyed his qualifying exam, simply because it was fun to interact with someone who was so intellectually gifted and who had so much potential. I did not feel like I was examining Rob: rather, we were having an advanced technical discussion among equals.

Subsequent conversations with Rob only strengthened my opinion of him, and I truly believe that, had he not passed away, he would have gone on to complete the Ph.D. and would have distinguished himself in a research career. His untimely death was a true loss to everyone that knew him.

In summary, because of his demonstrated, outstanding capabilities, I strongly support his nomination for the Ph.D. degree, posthumously.

Sincerely,

C.R. Ethier  
Associate Professor
November 19, 1996

Professor D.E. Cormack
Associate Dean - Division III
School of Graduate Studies
University of Toronto
65 St. George Street

Dear Professor Cormack:

Re: Mr. R.M. Prokop's Ph.D. Program

This letter is in strong support for the conferment of the Ph.D. degree on Robert Michael Prokop posthumously.

I write to you as the Graduate Studies Coordinator of our department, as well as a professor who has supervised many Ph.D. students. I can confidently state that, upon the completion of his degree requirements in the University of Toronto, Rob would have received offers for an academic position from numerous prestigious research universities in North America. His untimely death is thus a great loss to our scientific community.

Rob was an NSERC scholar and had always ranked top 5% during his graduate studies. This impeccable academic performance combined with his outstanding aptitude for research made him a great scientist. His publication record is a true reflection of his achievements, which are well beyond the norm. One can easily note the leadership role of Rob in most of these publications, as well as his significant contributions to his colleagues' research in the others.

In conclusion, it is an honour to recommend that the well-deserved degree of Doctor of Philosophy be conferred on Robert Michael Prokop posthumously.

Yours sincerely,

B. Benhabib
Professor and Associate Chair
November 20, 1996

Professor I. G. Currie
Chair
Department of Mechanical & Industrial Engineering
5 King's College Road
University of Toronto

Dear Professor Currie

Subject: Posthumous Awarding of the Ph.D. Degree to Mr. Rob M. Prokop

Rob Prokop was indeed a sparkling talent with an inquiring mind. He reached out and inspired many of his young colleagues and friends by his dedication and example. He was a true scholar and we are all the poorer for his sudden passing.

I write to express my strong support for the awarding of the degree of Ph.D. on Rob Prokop posthumously. I have reviewed the published materials and am very impressed by the calibre of the work and the external assessments which preceded publication. Others too will comment on this work which therefore allows me the opportunity to share other aspects of the character of the individual.

Rob Prokop chose to work under the supervision of Professor Wilhelm Neumann in the Department of Mechanical Engineering. Professor Neumann, supervisor of many Ph.D. and Masters theses over the past 25 years, maintains impressive laboratory facilities to support his innovative research into the understanding of the intricacies that make up the science and engineering of surface thermodynamics. His work is well supported through both MRC and NSERC. More important than the infrastructure and facilities, is the environment that is created by Professor Neumann in which his students are encouraged to extend their horizons to new high as confirmed by the work of Rob Prokop and his insights into the modeling applicable to lung surfactants and other systems. In this context it is significant to note that Professor Neumann recently received the prestigious Northrop Frye Award.

It is clear, that Rob Prokop, while registered as a Ph.D. student accomplished a great deal, contributed extensively to the published literature, and was on a fast track to complete a very significant thesis encompassing elements of design, computing and biotechnology and directed to
the modelling of lung surfactant systems. What I wish to share with you is the impact of his leadership on his graduate colleagues as well as the many undergraduates that have worked in the surface thermodynamics laboratories. Some ten days following his unexpected death, colleagues and co-workers of Rob Prokop arranged for a commemorative service held in the Wallace Room of the Department of Mechanical and Industrial Engineering. This service was attended by Rob's parents and friends of Rob within the Department. I wanted to attend and was very pleased that I did. Two co-authors of Rob, Ajay Iyoti and Pu Chen, addressed the group and shared with us Rob's commitment and dedication to his work, his excitement and the quiet, supportive leadership that he provided. It was a difficult afternoon, but one that made me very proud to be associated with the graduate students within the Department of Mechanical and Industrial Engineering. Rob's parents were both in attendance and really valued the outpouring of this affection and true recognition of their son's efforts.

Last weekend I had the opportunity to attend the annual dinner of the mechanical and industrial undergraduate students. Quite unexpectedly I got chatting to a group of third year mechanical engineering students. As the conversation progressed I learnt that five of these students had actually worked in the Neumann laboratories during the summer of 1996 on specific projects supervised by Rob Prokop. I was immediately impressed by their collective praise for his work and efforts on their behalf. They all had enjoyed working with him: he taught us a great deal and was always so approachable and understanding.

It is increasingly evident to me that Rob Prokop was not only a bright talent that excelled in his research endeavours, but that he was able to inspire his colleagues and to motivate them with a sense of purpose and direction. He is missed by all of them and life in the Neumann laboratories is now different for everyone. Yet Rob has left his mark and many will learn and follow his example.

We will never know the full meaning of the potential of Rob Prokop. In his years as a Ph.D candidate, working within the Neumann laboratories, he excelled in his research and was truly respected by his peers, professors and fellow students. It is very important to recognize his contributions and to award the Ph.D posthumously. This small gesture, so well earned, will give comfort to his family and friends. They deserve no less.

Yours sincerely,

Ron D. Venter
Vice-Dean and
Clarice Chalmers Professor
of Engineering Design
Memorial Speech delivered in the Wallace Room of the Mechanical Engineering Building in honour of Robert Prokop, by Ajay Jyoti on July 5, 1996.

It was in this room, just under two years ago, that I first met Rob. It was the Mechanical Engineering Graduate Student Association orientation session, and it was my first exposure to Rob's logical deduction and sense of humour.

Rob had walked straight up to me and asked, "You're going to be working with Prof. Neumann, right?". I had no idea who this was, much less how he knew who I would be doing my post-graduate work with. Dumbfounded, I said nothing. Rob, enjoying every minute of this, continued: "You just graduate from McGill University, right?" I nodded dumbly. "You spent this summer travelling throughout Europe, didn't you?" This continued for little while, during which I just stood, staring at this stranger who knew all about me.

It turns out that Rob had read my name-tag, and had recognized me as the other student who would be working with him in the Laboratory for Applied Surface Thermodynamics. Rob had learned some information about me from Prof. Neumann.

That was the start of our friendship.

We immediately grew close because we were both new to the University of Toronto and new to the city. We would spend evenings and weekends trying to get our experiments to work. Although these times were serious, they were always made more enjoyable by Rob's storytelling and sense of humour.

That first winter, Rob spent Christmas time with me, my family and my friends in Montreal. Everyone there took an instant liking towards him.

Those of us who are here today, whether we are his undergraduate students, fellow graduate students, or his professors, know of his brilliance, determination, and good-naturedness.

I remember when we first joined the lab and Rob was working hard on a theory relating equations of state to monolayers. It was only when he had his working model nearly complete did a colleague point out that this had been done previously. Rob was slightly disappointed, but looked on the bright side: "At least I'm on the right track," he had said. Unwavering, he started on something new.

Rob's lung model, which eloquently looks at the functioning of the lung from a mechanical engineer's point of view, was the result of his perseverance.

Rob's talent was not limited to academics, nor did he relate only to adults.

Pu Chen, a colleague of ours, tells of a story of how his son Patrick's first steps were in Rob's presence. Rob, of course, took full credit for this.
Rob worked effectively with people of all ages: he had the patience to teach diving to elementary school children at the University of Toronto Athletic Centre. He also had the patience to teach Differential Equations to undergraduate mechanical engineering students. Judging from the response he received when he asked for volunteers to work in the lab, those students were very fond of Rob. I remember him walking back from every class with a handful of resumes. Some of those students are here today.

Rob touched our lives with his brilliance, his good-naturedness and his sense of humour. Through him we learned and grew.

We will always cherish these memories and remember what he brought us. In this way, Rob will be with us always.
Memorial Speech: in honour of Rob Prokop

by Pu Chen

July 5, 1996

The news of Rob’s death on June 18, 1996 shocked us and made us extremely sad. We lost not only a very good friend but also a brilliant scientist. I remember how Rob and I quickly became close after we first met because we shared many things in common about science, friendship and opinions on world affairs. We soon started to have lunch together in this common room (Wallace room of Mech. Building), along with Ajay. We often joked through our lunch. An important part of this lunch break was the after-lunch chat: we made numerous “inventions”. One time, we resolved a key problem of the pulsating bubble technique, a technique which is often used in surface tension measurements. We immediately agreed this would make us a fortune, and we planned to give up academic matters and go into business. Once we were financially secured, science could become our hobby.

Just two days ago, when I came here for my lunch, I felt an enormous emptiness. At the same time, I realized the friendship between Rob and me will be one for life.

Rob was a very talented man, a man with diversity. He biked across Australia, bungy jumped in New Zealand, and even planned to go scuba diving in Israel. Not only did he like all these, but also did well in each of them. His interest in diving led him to become a diving teacher. He taught a diving class for kids in the Athletic Centre of University of Toronto, and he enjoyed every bit of it.

Relating to kids, I also remember how Rob made my son, little Patrick, walk for the first time. The story has been stolen by Ajay, and he just told this to you.

In research, Rob was not only brilliant but also creative. An example is seen in his lung modelling. A part of his thesis is to study lung surfactants; however, because the lung is such a complicated system, many properties have to be known before one can understand anything about it. A correlation among these properties immediately becomes an issue. Numerous attempts have been made, but little success has been achieved. However, the genius of Rob has made substantial progress through an ingenious combination of thermodynamics and lung mechanics. Perhaps, one thinks this is no big deal and the combination is a natural thing for any biomedical engineer. To me, Rob’s contribution is very remarkable. We all know thermodynamics, the equilibrium conditions and the stability conditions; but, for the past 50 years, no one had imagined these simple thermodynamic concepts could do wonders in lung modelling. Only when Rob started working on this, the lung modelling became anchored in thermodynamic principles, allowing the properties of the lung to be correlated with one another.

Rob’s talent in science is rooted both in his creativity and his perseverance. I remember from his early stages of developing the lung model that his biggest progress was made after a sleepless night. The next day I thought he would go back home early, but to my surprise, he stayed in the lab even later than usual because he was so excited about his discovery and tiredness could not slow him down.

Through his short time with us, we experienced so much of Rob: his humanity, his brilliance, his creativity, and of course, his friendship. Let us remember these, remember Rob Prokop. Thank you.
ROBERT PROKOP

PERMANENT ADDRESS:
422 - 52313 Rge. Rd. 232
Sherwood Park. AB. CANADA
T8B 1B7
Phone: Home: (416) 653-9148
Office: (416) 978-1270

CURRENT OBJECTIVE:
Doctor of Philosophy in Mechanical Engineering
University of Toronto. Toronto. ON
~ Cumulative G.P.A.: A+

EDUCATIONAL ACHIEVEMENTS:
Master of Science in Mechanical Engineering
University of Alberta. Edmonton. AB. 1993
~ Graduating G.P.A.: 8.8/9.0
~ Class Rank: 2/123
Bachelor of Science in Mechanical Engineering (Cooperative Program) with Distinction
University of Alberta. Edmonton. AB. 1992
~ Graduating G.P.A.: 8.3/9.0
~ Class rank: 3/86

~ Natural Science and Engineering Research Council (NSERC) Postgraduate Scholarship (1992-95)
~ Walter H. Johns Graduate Fellowship (1992-93)
~ Natural Science and Engineering Research Council (NSERC) Summer Research Award (1992)
~ Luscar Engineering Bursary in Mechanical Engineering (1991)
~ Kieran Hayden Memorial Award in Mechanical Engineering (1990)
~ Norcen Energy Resources Scholarship (1989)
~ Alexander Rutherford Scholarship (1987)
~ S.P.C.S.S. Academic Excellence Award (1987)
~ Province of Alberta Matriculation Scholarship (1987)

MAJOR PUBLICATIONS:
CONFERENCE PRESENTATIONS:


EMPLOYMENT HISTORY:

September 1994 - Present
Dr. A.W. Neumann, University of Toronto, Toronto, ON
Research Assistant

- developed methodologies and carried out experiments on the interfacial properties of pulmonary surfactant and insoluble monolayers.
- supervised three undergraduate research assistants.
- assisted in the refereeing of journal publications and grant applications.

January 1995 - May 1995
Dept. of Mechanical Engineering, University of Toronto
Lecturer

- lectured in an undergraduate differential equations course.

July 1993 - September 1994
Dr. W.H. Finlay, University of Alberta, Edmonton, AB
Research Assistant

- set up the experimental apparatus and collected data for a study of regional lung deposition of hygroscopic medical aerosols.
- developed a methodology for testing vented ultrasonic nebulizers.
September 1992 - May 1993
Dept. of Mechanical Engineering, University of Alberta, Edmonton, AB
Graduate Teaching Assistant

- prepared and gave laboratory lectures and demonstrations, made and marked assignments, and
  provided consultation for undergraduate fluid mechanics, dynamics, and technical report writing
  courses.

May 1992 - August 1992
Dr. W.H. Finlay, University of Alberta, Edmonton, AB
Summer Research Assistant

- made modifications to an existing Fortran computer code for stability analysis of rotating boundary
  layer fluid flow.

January 1991 - August 1991
BP Resources Canada Limited, Calgary, AB
Co-op Operations Engineer

- evaluated the performance of equipment and made recommendations about future installations.
- carried out an optimization study for chemical usage.

May 1990 - August 1990
Alberta Power Limited, Battle River Generating Station, AB
Co-op Mechanical Engineer

- designed plant modifications and supervised their installations.
- programmed and ran computer models and simulations.

September 1989 - December 1989
Nova Corporation of Alberta, Calgary, AB
Co-op Design Engineer

- sized HVAC equipment, heat exchangers, pumps, valves, and piping.
- carried out investigative and feasibility studies.
- programmed and ran computer models and simulations.

May 1989 - August 1989
Esso Resources Canada Ltd., Judy Creek Gas Conservation Plant, AB
Assistant Mechanic

- assisted in maintenance checks and repairs of turbines, compressors, pumps, and coolers: repaired
  and installed compressor valves.

ADDITIONAL INFORMATION:

Organized Extra-curricular Activities:
- Treasurer, Mechanical Engineering Graduate Students' Association (1995)
- Toronto Bicycle Network (1995-95)
- University of Toronto Springboard Diving Club (1995-95)
- University of Alberta Squash Club (1991-93)
- University of Alberta Dance Club (1990-91)
Hobbies:
- downhill skiing, swimming, scuba diving, cycling, and reading

REFERENCES:
Available on request
LIST OF PhD RELATED PUBLICATIONS - ROBERT M. PROKOP
(AS OF NOVEMBER 1996)


LIST OF PhD RELATED CONFERENCE PAPERS - ROBERT M. PROKOP
(AS OF NOVEMBER 1996)


Ph.D. Related

Publication #1
Interfacial Tension from the Height and Diameter of Sessile Drops and Captive Bubbles with an Arbitrary Contact Angle

R. M. PROKOP, O. L. del RIO, N. YIYAKAN and A. W. NEUMANN

Department of Mechanical Engineering, University of Toronto, 5 King's College Road, Toronto, ON MSS 3G8

A method has been developed to calculate the interfacial tension of sessile drops and captive bubbles of arbitrary contact angle by measuring the drop diameter and vertical distance to the apex at horizontal planes within the drop. The procedure works in theory for any contact angle with an accuracy on the order of 0.1%. However, practical limitations reduce the range of angles to roughly 50°-180° but do not restrict the range of interfacial tensions (at least 0.01 mJ/m² to 72.0 mJ/m²). The optimal strategy is to use the method at several points on a single drop and to calculate the mean and standard deviation of the resulting interfacial tensions.

On a mis au point une méthode dans le but de calculer la tension interfaciale de gouttes sessiles et de bulles captives ayant un angle de contact arbitraire en mesurant le diamètre des gouttes et la distance verticale jusqu'au sommet pour des plans horizontaux arbitraires à l'intérieur de la goutte. La méthode fonctionne en théorie pour n'importe quel angle de contact avec une précision de l'ordre de 0.1%. Cependant, les limites pratiques réduisent la gamme des angles à environ 50-180° mais sans restreindre la gamme des tensions interfaciales (au moins de 0.01 mJ/m² à 72.0 mJ/m²). La stratégie optimale consiste à appliquer la méthode à différents points sur une seule goutte et de calculer l'écart moyen et l'écart-type des tensions interfaciales résultantes.

Keywords: surface tension, interfacial tension, sessile drop, captive bubble.

It has been known for over a century that the interfacial tension of a sessile drop or captive bubble may be computed from the drop dimensions. Early methods (c.f. Quincke, 1859; Worthington, 1885; Ferguson, 1913; Turner, 1921; Porter, 1933; Padday and Pitt, 1972) are generally deficient in that they require either the height of the drop above the equatorial diameter (which may be difficult to locate precisely) or the maximum height achieved by a growing drop (which may not be obtainable if the sample volume is limited).

To avoid these difficulties, the method of Malcolm and Elliott (1980) used the total height $H$ and the maximum diameter $D$ of a sessile drop or captive bubble with a 180° contact angle to calculate the interfacial tension. (Since a captive bubble is equivalent to an inverted sessile drop, the term “drop” will be used in the remainder of this paper to refer to both geometries.) Like Padday (1971) and Bashford and Adams (1883), they used $R_o$, the radius of curvature at the apex of the drop to normalize the drop dimensions. Hartland and Hartley (1976) used the capillary length $a$ to normalize the drop dimensions: conversion from one set of data to the other is possible by using the relations

$$a = \left(\frac{\gamma}{\Delta \rho g}\right)^{1/2} \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \Quad
\[ \frac{dx}{ds} = \cos \phi \]  
\[ \frac{d\phi}{ds} = \frac{b + c - \sin \phi}{r} \]  
\[ \frac{dz}{ds} = \sin \phi \]

Here \( \phi \) is the angle between the tangent to the interface and the horizontal (see Figure 1). \( s \) is the arc length measured from the apex of the drop, \( b \) is the curvature at the apex of the drop (i.e., \( b = 1/R_0 \)), and \( c \) is the capillary constant defined by

\[ c = \frac{\Delta \phi \beta}{\gamma} \]

The boundary conditions at \( s = 0 \) are given by

\[ x(0) = z(0) = \phi(0) = 0 \]

and

\[ \frac{dx}{ds} = b \]

For given values of \( b \) and \( c \), the above set of equations can be used to determine \( x, z, \) and \( \phi \) as functions of \( s \). Since there is no known analytical solution for this set of equations except for very limited cases, a computer code was developed to solve the equations numerically (del Rio, 1993). The program calculates interfacial coordinate points and inclination angles for an input step size \( \Delta s \) using Bulirsch-Stoer integration (Press et al., 1992). The precision of the resulting coordinate points is unaffected by the step size and is always set to \( 10^{-14} \). The equations are integrated up to a specified value of \( s \) or until \( \phi = 180^\circ \).

Another computer code, into which a fixed value of the inclination angle \( \phi \) and a range of \( \beta \) values are provided as input, was linked to the first program. The coordinate points for drops for each value of \( \beta \) were computed to obtain the values of \( H_s/D_0 \) and the values of \( G(\phi, H_s/D_0) \) were subsequently calculated from Equations (1) and (8). This procedure was completed for \( 0^\circ \leq \phi \leq 180^\circ \) in \( 10^\circ \) increments and the resulting values of \( G(\phi, H_s/D_0) \) are plotted in Figure 2.

**EVALUATION OF THE METHOD**

In order to test our method of calculating the interfacial tension, four sets of drop profile coordinates were obtained by numerically solving Equations (10)-(12) in the manner discussed above. The values of \( b \) and \( c \) for each of the test drops is shown in Table 1 and the resulting drop profiles are shown in Figure 3. These values were selected to demonstrate that the present methodology is applicable even at the low interfacial tensions that can be generated in pulmonary surfactant by compressed captive bubbles (c.f., Schurch et al., 1989, 1992 and

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**Figure 1** — The geometry of a general sessile drop. \( H \) and \( D \) correspond to the required measurements of height and diameter for the method of Malcolm and Elliott (1980). \( H_s \) and \( D_s \) correspond to the height and diameter at the point where the inclination angle is equal to \( \phi \).

\[
D_s = 2x_s \quad H_s = z_s \quad 0^\circ \leq \phi < 90^\circ \\
D_s = 2x_0 \quad H_s = z_s \quad 90^\circ \leq \phi \leq 180^\circ 
\]

Here the bars denote quantities that have been normalized by the radius of curvature \( R_0 \). Note that \( D_s = D \) and \( H_s = H \) when \( \phi = 180^\circ \). However, in general, the inclination angle \( \phi \) does not have to cover the range from \( 0^\circ \) to \( 180^\circ \). For a given fluid of surface tension \( \gamma \), one can simultaneously vary the drop volume and the type of solid substrate in such a way that the drop profiles are identical over some range of \( \phi \). Therefore, the range of \( \phi \) depends on the solid substrate material and drop volume, but not on the capillary length \( a \) or radius of curvature \( R_0 \) of the drop. A drop such as the one shown in Figure 1 is the most general form since \( \phi \) ranges from \( 0^\circ \) to \( 180^\circ \); drops of any contact angle can be generated from these data by terminating the drop profile at the appropriate point.

In a manner analogous to that of Malcolm and Elliott (1980), define a function \( G(\phi, H_s/D_0) \) such that

\[
a = \frac{H_s}{G(\phi, H_s/D_0)}
\]

By substituting Equation (8) into Equation (1), the following expression is obtained:

\[
\gamma = \Delta \phi \left( \frac{H_s}{G(\phi, H_s/D_0)} \right)^2
\]

Thus, once the functional form of \( G(\phi, H_s/D_0) \) is known, the interfacial tension of the drop can be computed at any location along the drop profile from Equation (9). For \( 90^\circ \leq \phi \leq 180^\circ \), this procedure is similar to that of Coucoulaus and Dawe (1985). However, the present method is more general since any inclination angle between \( 0^\circ \) and \( 180^\circ \) may be used to compute the interfacial tension.

**Results and discussion**

**DETERMINATION OF \( G(\phi, H_s/D_0) \)**

The profile of an axisymmetric Laplacian sessile drop may be expressed by the following set of first order differential equations (Rotenberg et al., 1983):

\[
\frac{dx}{ds} = \cos \phi \\
\frac{d\phi}{ds} = \frac{b + c - \sin \phi}{r} \\
\frac{dz}{ds} = \sin \phi
\]
The use of tables to compute the interfacial tensions from the drop dimensions is of limited practical use since one would require an extensive set of tables for \( G(\alpha, H_o/D_o) \) versus \( H_o/D_o \) for numerous inclination angles. To avoid this shortcoming, a fourth order polynomial was fit to \( G(\alpha, H_o/D_o) \) for each value of \( \alpha \) such that

\[
G(\alpha, H_o/D_o) = A_0 + A_1(\alpha, H_o/D_o) + A_2(\alpha, H_o/D_o)^2 + A_3(\alpha, H_o/D_o)^3 + A_4(\alpha, H_o/D_o)^4
\]

The resulting coefficients are shown in Table 3 and the correlation coefficients and the errors in the polynomial fits are shown in Table 4. Note that \( G(\alpha, H_o/D_o) \) becomes a very steep function for small values of \( \alpha \), therefore the range of \( \beta \) was gradually decreased to maintain the errors at an approximately constant level. Over most of the range of inclination angles, the average errors in the polynomial fits are so small that they are negligible.

In order to use the polynomials at an arbitrary value of \( \alpha \), it was necessary to devise a scheme to interpolate the polynomial coefficients. It was found that it was not practical to fit polynomials of the type shown in Equation (16) to all of the coefficient data since the coefficient functions become extremely steep at small inclination angles and very high order polynomials would be required. Instead, a computer program was developed that uses Neville's algorithm (Press et al., 1992) to fit a polynomial of degree \( N-1 \) to the \( N \) known points nearest the desired value. This algorithm is particularly useful since it automatically keeps the approximations centred as much as possible on the desired value.

<table>
<thead>
<tr>
<th>Drop</th>
<th>( \gamma (\text{mJ/m}^2) )</th>
<th>( h(\text{cm}) )</th>
<th>( \ell(\text{cm}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>22.0</td>
<td>0.25</td>
<td>13.472</td>
</tr>
<tr>
<td>B</td>
<td>30.0</td>
<td>0.5</td>
<td>32.333</td>
</tr>
<tr>
<td>C</td>
<td>0.100</td>
<td>0.0</td>
<td>0.0000</td>
</tr>
<tr>
<td>D</td>
<td>0.0100</td>
<td>0.05</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

The variation of the function \( G(\alpha, H_o/D_o) \) with the angle of inclination \( \alpha \) and the height to diameter ratio \( H_o/D_o \) obtained by numerical integration of the Laplace equation. Similar curves were generated for \( 10^\circ \leq \alpha \leq 180^\circ \) in \( 10^\circ \) increments, but for clarity in the figure, the curves are plotted in \( 30^\circ \) increments.

1994), or in other systems with ultra-low interfacial tensions (Kwok et al., 1993). The use of the present method on ultra-low interfacial tensions is of particular significance since it is applied to a stationary drop and is therefore more easily implemented than other techniques such as the spinning drop method.

Using the test drop profile coordinates and the tables of \( G(\alpha, H_o/D_o) \) versus \( H_o/D_o \) computed in the manner described earlier, the results shown in Table 2 were obtained. These results clearly demonstrate that the present methodology works over a broad range of inclination angles and interfacial tensions. The method of Malcolm and Elliott (1980) is only applicable to drops with a contact angle of \( 180^\circ \), while the method of Coucoulias and Dawe (1985) is limited to \( 90^\circ \leq \alpha \leq 180^\circ \). Therefore, the present method is clearly an improvement over both of these methods since it is applicable to any drop. In addition, it should be noted that when \( \alpha = 90^\circ \), one is measuring the same drop dimensions as are required for the method of Padday and Pitt (1972).

![Figure 2](image-url)  

**Figure 2** — The variation of the function \( G(\alpha, H_o/D_o) \) with the angle of inclination \( \alpha \) and the height to diameter ratio \( H_o/D_o \) obtained by numerical integration of the Laplace equation. Similar curves were generated for \( 10^\circ \leq \alpha \leq 180^\circ \) in \( 10^\circ \) increments, but for clarity in the figure, the curves are plotted in \( 30^\circ \) increments.

![Figure 3](image-url)  

**Figure 3** — Drop profile coordinates for four test drops generated by numerical integration of the Laplace equation. The input values of the curvature \( b \) and the capillary constant \( c \) for each drop are listed in Table 1. The origin of the coordinate system is at the apex of each drop.
Obtained by linear interpolation of the $G(\theta, H_x D_y)$ vs. $H_x D_y$ that was calculated by numerically integrating the Laplace equation.

<table>
<thead>
<tr>
<th>Inclination</th>
<th>Range of $H_x D_y$</th>
<th>Range of $H_y D_z$</th>
<th>$A_{1,0}$</th>
<th>$A_{1,1}$</th>
<th>$A_{1,2}$</th>
<th>$A_{1,3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>180°</td>
<td>0.2 - 10.0</td>
<td>1.56 - 0.87</td>
<td>1.290632</td>
<td>1.0737135</td>
<td>-10.669259</td>
<td>9.2427394</td>
</tr>
<tr>
<td>170°</td>
<td>0.2 - 10.0</td>
<td>1.51 - 0.83</td>
<td>1.215656</td>
<td>1.087970</td>
<td>-10.145889</td>
<td>9.3566984</td>
</tr>
<tr>
<td>160°</td>
<td>0.2 - 10.0</td>
<td>1.49 - 0.80</td>
<td>1.791452</td>
<td>1.342163</td>
<td>-10.440345</td>
<td>9.8288223</td>
</tr>
<tr>
<td>150°</td>
<td>0.2 - 10.0</td>
<td>1.47 - 0.83</td>
<td>1.758578</td>
<td>1.257258</td>
<td>-1.044996</td>
<td>10.800668</td>
</tr>
<tr>
<td>140°</td>
<td>0.2 - 10.0</td>
<td>1.43 - 0.79</td>
<td>1.697810</td>
<td>7.416340</td>
<td>1.993142</td>
<td>12.421319</td>
</tr>
<tr>
<td>130°</td>
<td>0.2 - 10.0</td>
<td>1.38 - 0.75</td>
<td>1.623387</td>
<td>7.221925</td>
<td>13.401311</td>
<td>14.990475</td>
</tr>
<tr>
<td>120°</td>
<td>0.2 - 10.0</td>
<td>1.32 - 0.69</td>
<td>1.532176</td>
<td>7.955356</td>
<td>15.500035</td>
<td>19.170114</td>
</tr>
<tr>
<td>110°</td>
<td>0.2 - 10.0</td>
<td>1.25 - 0.62</td>
<td>1.422927</td>
<td>8.429030</td>
<td>-18.73030</td>
<td>26.486451</td>
</tr>
<tr>
<td>100°</td>
<td>0.2 - 10.0</td>
<td>1.17 - 0.57</td>
<td>1.292081</td>
<td>5.158921</td>
<td>-24.384927</td>
<td>40.809923</td>
</tr>
<tr>
<td>90°</td>
<td>0.2 - 10.0</td>
<td>1.09 - 0.48</td>
<td>1.130624</td>
<td>6.447894</td>
<td>-35.253999</td>
<td>73.383935</td>
</tr>
<tr>
<td>80°</td>
<td>0.2 - 10.0</td>
<td>0.99 - 0.47</td>
<td>0.938711</td>
<td>3.530723</td>
<td>-55.416843</td>
<td>145.33769</td>
</tr>
<tr>
<td>70°</td>
<td>0.2 - 10.0</td>
<td>0.89 - 0.42</td>
<td>0.732179</td>
<td>11.347268</td>
<td>-83.354006</td>
<td>287.5185</td>
</tr>
<tr>
<td>60°</td>
<td>0.2 - 10.0</td>
<td>0.79 - 0.28</td>
<td>0.598909</td>
<td>15.298592</td>
<td>-144.53069</td>
<td>581.70158</td>
</tr>
<tr>
<td>50°</td>
<td>0.4 - 10.0</td>
<td>0.68 - 0.22</td>
<td>0.379952</td>
<td>17.373230</td>
<td>-201.28921</td>
<td>1007.5925</td>
</tr>
<tr>
<td>40°</td>
<td>1.0 - 10.0</td>
<td>0.56 - 0.17</td>
<td>0.272222</td>
<td>19.008188</td>
<td>-276.83009</td>
<td>1757.8796</td>
</tr>
<tr>
<td>30°</td>
<td>2.0 - 10.0</td>
<td>0.44 - 0.13</td>
<td>0.129498</td>
<td>23.221181</td>
<td>-451.04142</td>
<td>3835.5007</td>
</tr>
<tr>
<td>20°</td>
<td>4.0 - 10.0</td>
<td>0.32 - 0.08</td>
<td>0.055452</td>
<td>29.547010</td>
<td>-854.11036</td>
<td>10842.191</td>
</tr>
<tr>
<td>10°</td>
<td>10.0 - 10.0</td>
<td>0.18 - 0.04</td>
<td>-0.297483</td>
<td>3.004006</td>
<td>-4001.9283</td>
<td>95949.998</td>
</tr>
</tbody>
</table>

The errors of the greatest magnitude occur when near the largest $H_y D_z$ for a given value of the inclination angle $\theta$. In all cases, the correlation coefficient $R \approx 0.999$.

and can be used for any value of $N$ less than or equal to the total number of points. Figure 4 shows the resulting calculated values of the interfacial tension for the four test drops for each of five values of $N$. As can be seen in the figure, the amount of error in the interfacial tension increases as the inclination angle decreases. This is due to the fact that the polynomial coefficients become extremely steep functions of the inclination angle for small inclination angles. Note that in all cases, the value of $N = 8$ provides the broadest range of inclination angles for a given amount of error in interfacial tension. In Table 5, the ranges of inclination angles are shown for fixed values of the maximum error in interfacial tension for the four test drops.

**Sensitivity Analysis**

To simulate the effect of experimental measurement errors on the procedure, perturbations were introduced to the drop profile coordinates and the inclination angles of the four test drops. Performing the evaluation in this manner is important for two reasons: First, it allows one to clearly differentiate between the errors in the method itself and the errors due to experimental measurement errors. Second, using this method, one can obtain an error estimate as a percentage of the true interfacial tension (which may not be known from experimental data due to experimental errors). Furthermore, although Coucouulas and Dawe (1985) performed a differential sensitivity analysis on their method, this procedure does not allow one to easily estimate the actual magnitude of the error in interfacial tension; only a relative error would be known.
The Range of Inclination Angles for a Given Maximum Error in Interfacial Tension for the Four Test Drops

<table>
<thead>
<tr>
<th>Maximum Error</th>
<th>Drop A</th>
<th>Drop B</th>
<th>Drop C</th>
<th>Drop D</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>46°-180°</td>
<td>50°-180°</td>
<td>46°-180°</td>
<td>50°-180°</td>
</tr>
<tr>
<td>1%</td>
<td>55°-180°</td>
<td>60°-180°</td>
<td>53°-180°</td>
<td>59°-180°</td>
</tr>
<tr>
<td>0.5%</td>
<td>85°-180°</td>
<td>95°-180°</td>
<td>85°-180°</td>
<td></td>
</tr>
</tbody>
</table>

It was assumed that the profile coordinates of an experimental drop would be obtained from a digitized image of the drop using an edge detection scheme. In Figure 5, all drop profile coordinates were perturbed by an amount \( \delta \) corresponding to 1 pixel on a 640 \( \times \) 480 digitized image of the drop, i.e.,

\[
\delta = \frac{1}{640} D_{90}
\]  

As can be seen in the figure, the perturbations introduce an error of about 5% at small inclination angles; the error decreases as the inclination angle increases.

In Figure 6, the results of perturbing the inclination angles by 1° are shown. It is clear that the calculated surface tension is most sensitive for small inclination angles, but has a maximum value of about 5% for all four interfacial tensions.

To simulate more closely the use of our method on experimental data, the drop profile coordinates were perturbed randomly with a maximum value corresponding to \( \pm 3 \) pixels. As can be seen in Figure 7, the random fluctuations cause random fluctuations in the interfacial tension about the true value.

Figure 8 simulates the use of the method of Malcolm and Elliott (1980) on drops with contact angle of less than 180°. Although Malcolm and Elliott (1980) stressed that their method is only applicable for contact angles of less than 180°, it is difficult to discern high values of the contact angle in an experiment, which may result in an error in the interfacial tension.
tension calculation. For example, if the method of Malcolm and Elliott (1980) is applied to a drop with an actual contact angle of 150°, an error of about 10% in the interfacial tension would result.

APPLICATION OF THE METHOD

There are several aspects of the physical set-up of an experiment that require consideration when applying the method of measuring interfacial tension described in this paper. If possible, the solid substrate should be selected so as to maximize the contact angle, thereby increasing the range of possible inclination angles. For a sessile drop, this means selecting a hydrophobic material, while for a captive bubble, a hydrophilic material is required. However, because the contact angle can be affected by both heterogeneity (Neumann and Good, 1972) and the roughness (Eick et al., 1975) of the solid, the use of very high inclination angles (i.e., points near the solid surface) is not recommended unless the solid substrate is known to be very smooth and homogeneous. This is particularly important when the size of the drop is varied to determine the relationship between surface area and interfacial tension, as is the case in investigations of pulmonary surfactant (Schürch et al., 1989, 1992 and 1994). Heterogeneity or roughness could potentially lead to contact angle hysteresis (Neumann and Good, 1972; Eick et al., 1975), so that very high inclination angles would not be reliable.

The volume of the drop is also an important consideration. As the volume of a drop of a given interfacial tension increases, the drop shape becomes less spherical. This effect is more pronounced for drops of low interfacial tension, in which case broad, flat drops with very low values of H/D are observed when the volume is increased. As discussed earlier, the accuracy of our method of measuring the interfacial tension decreases as the inclination angle decreases. Since the
The majority of the arc length along a broad, flat drop would have a low inclination angle, such drops would lead to less accurate measurements of the interfacial tension. Furthermore, a nearly spherical drop can be viewed with greater magnification since it would more closely fit into the image field than a broad, flat drop due to the fact that most image fields have an aspect ratio of roughly 1.5. As a result, the coordinate points and inclination angles can be measured with greater accuracy on an nearly spherical drop. Consequently, one should make the volume of the drop as small as possible in pulmonary surfactant systems or other systems with low interfacial tensions.

Taking the above discussion into account, the simulation of experimental measurement errors shown in Figure 7 implies that the optimal strategy for our methodology is as follows: Obtain an image of the drop and measure the values of $a$, $H_{ap}$ and $D_{ap}$ at several points along the drop profile. Compute the corresponding values of $a$, $H_{ap}$ and $D_{ap}$ from the coefficients listed in Table 3 and a suitable interpolation procedure such as Neville’s algorithm. Finally, substitute these values for each point into Equation (9) along with the density difference between the bulk phases and the local gravitational constant and then compute the mean and standard deviation of the resulting interfacial tensions. In this manner, the random errors introduced by the uncertainties in the drop profile coordinates and the values of the inclination angles would tend to average out. Although multiple measurements add some degree of complexity to the methodology, this strategy is desirable since the standard deviation would also provide an estimate of the quality of the calculated interfacial tension. Since the method of Malcolm and Elliott (1980) is based on a single measurement, there is no indication of the quality of the measurement.

Summary and conclusion

The method outlined in this paper allows one to calculate the interfacial tension of an arbitrary sessile drop or captive bubble at arbitrary points along the drop profile. It is based on the technique of Malcolm and Elliott (1980). Although the present method would work in principle for any inclination angle, practical limitations restrict the range of angles. However, the method works satisfactorily for interfacial tension at least as low as 0.01 mN/m and there is indeed no indication that the method might not be applicable for any interfacial tension. If desired, testing for other ranges of interfacial tension can be readily performed. While the present method is slightly more complicated than that of Malcolm and Elliott (1980), it can be used to provide an estimate of the quality of the measured interfacial tensions.

Acknowledgements

This work was supported through grant number A8278 of the Natural Sciences and Engineering Research Council (NSERC) and an NSERC Postgraduate Scholarship for R.M.P.

Nomenclature

- $a$: capillary length, m
- $b$: curvature at the apex of a drop, m
- $c$: capillarity constant, m
- $D$: maximum diameter of drop, m
- $D_{ap}$: diameter of drop at point of measurement, m
- $g$: acceleration due to gravity, m/s²
- $H$: total height of drop, m
- $H_{ap}$: vertical distance from point of measurement on drop profile to the apex of the drop, m
- $R_{ap}$: radius of curvature at the apex of the drop, m
- $s$: arc length along drop profile measured from drop apex, m
- $z$: normalized horizontal coordinate of drop profile point
- $z_{ap}$: vertical coordinate of drop profile point, m
- $z_{ap}$: normalized vertical coordinate of drop profile point
- $R_{ap}$: radius of curvature at the apex of the drop, m

Greek letters

- $\beta$: drop shape factor
- $\gamma$: interfacial tension, J/m²
- $\Delta\rho$: density difference between adjoining bulk phases, kg/m³
- $\theta$: inclination angle, degrees
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Manuscript received July 28, 1995; revised manuscript received January 29, 1996; accepted for publication February 21, 1996.
Ph.D. Related

Publication #2
Interfacial Tension of a 
Polystyrene–Poly(ethylene oxide) Diblock 
Copolymer at the Water–Toluene Interface

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Reprinted from
Volume 29, Number 18, Pages 5902–5906
Interfacial Tension of a Polystyrene—Poly(ethylene oxide) Diblock Copolymer at the Water—Toluene Interface

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Received February 13, 1996; Revised Manuscript Received June 12, 1996

ABSTRACT: This paper investigates the interfacial properties of a polystyrene—poly(ethylene oxide) (PS—PEO) diblock copolymer adsorbed at the water—toluene interface using axisymmetric drop shape analysis-profil (ADSA-P). The molecular weight of the PS block was 231 400 while the PEO block had a molecular weight of 20 670. The diblock copolymer was dissolved in toluene to form a 0.131 mg/mL solution and was allowed to adsorb onto a pendant drop of water at 20 °C. The variation in interfacial tension with time during the equilibration process was similar to that of a surfactant, beginning at the water—toluene interfacial tension and decreasing to an equilibrium value of 27.3 mJ/m² after a period of 2 h. The interface was then compressed by decreasing the volume of the drop in a linear manner. The shape of the resulting interfacial tension—area isotherm can be rationalized using a schematic model of the interfacial molecular conformation. According to the proposed model, the average molecular spacing is determined by the PS block at the start of compression and by a combination of the PEO block and the PS brush at the end of compression. These results show that the use of ADSA-P to measure interfacial tensions is a useful way to obtain indirect evidence of the interfacial molecular conformation.

Introduction

The surface activity of polymers is of industrial importance, and the adsorption of these materials at liquid—air and liquid—liquid interfaces has interested scientists for many years. In the early 1970s, Glass reported a series of experiments in which he extended the pendant drop technique to study the adsorption of a series of water-soluble polymers at a liquid—liquid interface. The interfacial tensions were computed from the shapes of pendant drops of water immersed in either hexane or benzene. Poly(ethylene oxide) (PEO) was among the water-soluble polymers studied at that time. It was a unique material since it is soluble in both water and aromatic solvents, and so the interfacial activity was studied by adsorption from both benzene and aqueous solution. In both types of experiments, the equilibrium interfacial tension was about 19 mJ/m² and there was little effect of molecular weight of the polymer.

More recently, electrocapillary wave diffraction techniques have been applied to polymers adsorbed at various liquid—liquid interfaces using Langmuir film techniques. Both PEO and polystyrene (PS) were investigated in various interfacial configurations: toluene/PEO in water; PEO in toluene/water; spread films of PEO at toluene/water; PS and PEO in toluene/water; and PS in toluene/PEO in water. Among other data, these measurements confirmed that PEO is highly surface active, that the limiting interfacial tension was about 19 mJ/m², and that the final adsorbed state was independent of the path by which equilibrium was reached. For the case of PS adsorbing from toluene at the toluene—water interface, the interfacial tension was shown to be the same as that of pure toluene—water (i.e., 36 mJ/m²), which indicates the likelihood of a depletion layer of PS at the interface similar to the PS depletion layer at the air—toluene interface. Over the past decade, the adsorption of diblock copolymers at interfaces has attracted attention both theoretically and experimentally. The theoretical predictions derived from scaling theories and mean field approximations have been examined using techniques such as direct force measurements, neutron reflectivity, and elliptometry. In all of these cases, the adsorption has been on to a solid surface, and the interpretations were based on the relative sizes of the two polymer components of the diblocks. However, there now appears to be some disagreement between theoretical predictions and experimental measurements. For example, recent work on the adsorption of diblock copolymers of poly(dimethylsiloxane)—polystyrene (PDMS—PS) at the air—ethyl benzate interface (using a Langmuir trough) concluded that current theories appear to underestimate the amount of energy required to stretch the diblock copolymer chains for this system. To determine the behavior of a diblock copolymer at a liquid—liquid interface, the interfacial tension of the toluene—water interface was investigated in the presence of a PS—PEO diblock copolymer. A modern derivative of the pendant drop technique, axisymmetric drop shape analysis-profile (ADSA-P), was used to measure the interfacial tension during the adsorption process and also during compression of the interface after equilibrium had been reached. These results can be interpreted through the use of a schematic model of the interfacial molecular conformation.

Experimental Section

Materials. The PS—PEO diblock copolymer (Polymer Laboratories, Church Stretton, UK) selected for this study contains PS with a molecular weight of 231 400 and PEO with a molecular weight of 20 670. The number of segments, N, associated with these molecular weights are 2225 and 470, respectively. A solution was obtained by dissolving 3.28 mg of the copolymer in 25 mL of toluene.

Since both blocks are soluble in toluene, we can define the asymmetry of the polymer (β) in terms of the Flory radii (Rf) of the equivalent polymers in solution:

\[
\beta = \frac{R_{F,PS}}{R_{F,PEO}}
\]
a tension coordinates or using then enm briefy, pmtiles with 256 pendant pendant drop of water; (2) Teflon capillary; (3) quartz cuvette; (4) environmental chamber; (5) water bath; (6) light source; (7) diffuser; (8) microscope; (9) CCD camera; (10) computer workstation; (11) syringe; (12) stepper motor; (13) stepper motor controller.

The Flory radius is obtained from the relation

\[ R_F = aN^{1/3} \]  \hspace{1cm} (2)

where \( a \) is the size of the individual polymer segments and \( N \) is the number of segments. The ratio \( a/a_0 \) is known from light scattering measurements to be 0.8713 so that the asymmetry of the copolymer used in this investigation is 2.21.

Measurement of the Interfacial Tension. A schematic diagram of the ADSA-P apparatus is shown in Figure 1. A pendant drop of doubly distilled water was formed on the end of a vertical Teflon capillary of circular cross-section. The volume of the drop was controlled by the stepper motor which was attached to the plunger of a syringe connected to the other end of the capillary. The drop was then lowered into a quartz cuvette that had been filled with the copolymer solution, and the cuvette was enclosed in an environmental chamber controlled to \( 20.0 \pm 0.1 \)°C by a thermostated water bath. The pendant drop was illuminated by a white light source shining through a heavily frosted diffuser. Using a microscope and a charge-coupled device (CCD) video camera, images of the pendant drop were digitized to an image of 640 x 480 pixels with 256 gray levels. The images were acquired from the digitization board by a Sun SPARCstation 10 workstation. The entire setup was mounted on a vibration table to minimize the effects of external vibrations.

The determination of the interfacial tension from the images of the pendant drops has been described in detail previously.\(^5\)Briefly, ADSA-P software determines the experimental drop profile coordinates to subpixel resolution. Theoretical drop profiles are computed from the Laplace equation of capillarity, and nonlinear regression techniques are used to minimize the deviation of the theoretical drop profiles from the experimental coordinates to calculate the interfacial tension, as well as the area, volume, and apex radius of curvature of the pendant drop.

It has been shown recently that ADSA-P can be used in a manner analogous to that of the classical film balance.\(^2\) By using the stepper motor and syringe to remove liquid from the drop, the surface area of the drop is decreased, and the change in interfacial tension can be plotted as a function of either time or drop surface area. For an insoluble film of octadecanol, there was excellent agreement between the results obtained by this new technique and those obtained with a traditional Langmuir trough.\(^4\) However, ADSA-P requires much smaller sample volumes than the Langmuir trough (the size of the drops used in this study was about 0.05 cm\(^2\)). Furthermore, since the current configuration of ADSA-P can acquire up to two images per second, it is much better suited for studying kinetic processes than the slowly responding Langmuir trough.

In the present set of experiments, the change in interfacial tension between the pendant drop of water and the toluene containing the PS-PEO diblock copolymer was monitored as a function of time during adsorption. The volume of the pendant drop was held constant during this process. Once equilibrium had been attained, the volume of the drop was reduced in a linear manner with time to attain a maximum area compression ratio \( A_{\text{final}}/A_{\text{initial}} \) of 0.20.

Results

The first step in our procedure was to determine the value of the water-toluene interfacial tension. The water-toluene interfacial tension was measured every 10 s for a period of 5 min for each of two runs. The error of each measurement was approximately 0.1 mJ/m\(^2\) and the mean value and 95% confidence limits for the resulting 90 measurements was \( 35.30 \pm 0.009 \) mJ/m\(^2\).

Figure 2 shows the interfacial tension between water and the copolymer-toluene solution as a function of time during the adsorption process on a pendant drop of constant volume. Measurements were made every 15 s for a period of 2 h. For each measurement, ten sets of twenty randomly selected profile points were used to compute the 95% confidence intervals of each data point. Note that the confidence intervals were generally of the order of 0.1 mJ/m\(^2\). During the last minute of the experiment, the interfacial tension was changing at an average rate of only \(-4.06 \times 10^{-4} \) mJ/m\(^2\)s. Therefore, the average interfacial tension of the last four data points, \( 27.80 = 0.02 \) mJ/m\(^2\), was taken to be the equilibrium value.

Once equilibrium had been reached, the volume of the pendant drop was decreased linearly over a period of 10 min to compress the interface. Strictly speaking, this should result in a nonlinear variation in area with time. However, for the range of drop sizes used in this study, the area compression rate was essentially linear. A linear regression on the data has a slope of \(-7.75 \times 10^{-4} \) cm\(^2\)/s with a linear correlation coefficient \( r = 0.997 \). After a compression to 20% of the initial area, the surface area was re-expanded by increasing the volume of the pendant drop. The resulting variations in volume and interfacial tension are shown as functions of time in Figure 3; the results are also plotted as interfacial tension versus area in Figure 4. On compression, the interfacial tension changes almost linearly from the initial value to about 25 mJ/m\(^2\). There is then a rapid decrease to about 15 mJ/m\(^2\), and finally the isotherm becomes almost vertical as the interfacial tension decreases to a final measured value of about 15 mJ/m\(^2\).

Since the accuracy of ADSA-P decreases with drop size, the lowest interfacial tensions are the least accurate.
Figure 3. Variation in drop volume and interfacial tension with time as the volume of the pendant drop is changed in a linear manner. The pendant drop was initially at equilibrium. The average compression rate was $-7.75 \times 10^{-3} \text{ cm}^2/\text{s}$.

Figure 4. Change in interfacial tension with area during compression and expansion. The letters B, C, and D correspond to different conformations of the interfacial molecules (see Figure 7).

It can also be seen that the process appears to be almost reversible when the drop is re-expanded: Although hysteresis of about 1 ml/m² is evident, the breaks in the $\gamma$-A isotherm occur at similar areas for compression and re-expansion.

A similar set of experiments was performed in which the average compression rate was $-1.14 \times 10^{-2} \text{ cm}^2/\text{s}$ ($r = 0.995$), which was 15 times faster than the previous set of experiments. The resulting interfacial tension--area isotherm is shown in Figure 5, while Figure 6 shows the effect of varying the compression ratio at a fixed compression rate. Four different compression ratios are shown. The overlap is remarkable, but once again it should be noted that the accuracy of the measurements decreases at very small areas. The general shapes of these isotherms are the same as Figure 4, but the break at 19 ml/m² is no longer obvious. The small amount of hysteresis for all the curves implies that there is no irreversible desorption from the interface.

Discussion

During the equilibration process, the adsorption of the diblock copolymer at the toluene--water interface results in a behavior that is similar to that of a surfactant. Both the PS and the PEO components are soluble in toluene, but as noted earlier, PS does not adsorb at the water--toluene interface and in fact probably forms a depletion layer. Therefore, any changes in the interfacial tension must be the result of the adsorption of the PEO into the interface.

During compression of the equilibrated interface, Figures 4--6 show that there is a decrease in interfacial tension with area. These results can be at least partially explained by considering the relative sizes of the two components of the diblock copolymer. The areas occupied by the PS and PEO blocks can be calculated from the Flory relationships:

$$\frac{A_{\text{PEO}}}{A_{\text{PS}}} = \frac{R_{\text{PEO}}^2}{R_{\text{PS}}^2} = \left(\frac{a_{\text{PEO}}}{a_{\text{PS}}}\right)^2 \left(\frac{N_{\text{PEO}}}{N_{\text{PS}}}\right)^{65}$$

where $A$ represents area and the subscripts denote the type of block. Note that this is almost identical to the maximum compression ratios which were possible to apply in the experiments, namely $A_{\text{final}}/A_{\text{initial}} = 0.20$.

To account for this result and to explain the shapes of the $\gamma$-A isotherm, a model of the conformational changes of the interfacial molecules is shown in schematic form in Figure 7. During adsorption (structure A in Figure 7), the diblock copolymer molecules move to the interface from the toluene. The PEO block adsorbs to the interface, while the PS block remains in the bulk toluene.

At the end of the equilibration process, there is a densely packed arrangement of the PS--PEO copolymer...
An intriguing paper by Kent et al. has addressed the energetics associated with the compression of the monolayer films of PS–PDMS block copolymers. Although their experiments were performed on monolayers at the air–liquid interface, their results are pertinent to the system described in this paper. They point out that the interfacial tension must include a term related to the stretching energy of the polymer brush (i.e., PS) and a conclusion from their arguments must be that when the PEO blocks just touch in the schematic of Figure 7, the interfacial tension must be lower than 19 mJ/m² by an amount equal to the stretching energy.

Using the ADSA-P methodology, we can confirm a value of the interfacial tension of homopolymer PEO at the water–toluene interface as being 18.4 mJ/m². In order to apply the comparative method employed by Kent et al., polymer adsorption would have to be assessed quantitatively. Some reasonable approximations of the surface density can be made by assuming that the PS blocks are just touching at the first equilibrium point (i.e., point B). Using the formulation by Kent et al., where occupied by one PS molecule (and therefore one copolymer molecule) is 11.51 × 10⁴ Å², which corresponds to a surface density of 8.7 × 10⁻⁶ molecules/Å². Assuming that the number of molecules at the interface is fixed, the data in Figure 4 were redrawn in Figure 8 to show the interfacial pressure generated as the polymer is stretched. It can be seen that the plot is almost linear. Unfortunately, interpretation and separation of the interfacial pressure components are not justified at this time and must await quantitative development of the adsorption isotherm of PEO at the same interface. Results from such experiments are nontrivial and will be discussed in a future publication.

**Summary and Conclusions**

The results obtained in this paper demonstrate that a PS–PEO diblock copolymer at the water–toluene interface behaves like a surfactant. Compression of the interface after equilibrium has been reached results in an interfacial tension–area isotherm that can be explained by considering the rearrangement of the diblock copolymer molecules at the interface. These results demonstrate that the interfacial tension measurements can be a useful way to provide indirect evidence of the conformation of the PS–PEO copolymer at the interface.

**Acknowledgment**

The authors acknowledge Dr. Paul Smith (XRCC) for his assistance in sample preparation, Janet Sze Mei Lam (UTME) for her assistance...
in performing the experiments, and the Natural Sciences and Engineering Research Council for a scholarship to support R.M.P. The authors also appreciate constructive comments made by the reviewers of the manuscript.

References and Notes


MA960230R
Ph.D. Related

Publication #3
Measurement of the interfacial properties of lung surfactant
Robert M Prokop and A Wilhelm Neumann

The classical methods used to investigate the surface activity of lung surfactant are the Langmuir—Wilhelmy surface balance and the pulsating bubble surfactometer. Over the past few years, it has become increasingly apparent that these methods are inadequate in many respects. These shortcomings, however, have been overcome by recent developments in lung surfactant methodologies: the captive bubble surfactometer and axisymmetric drop shape analysis.

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Current Opinion in Colloid & Interface Science 1996 1 677–681
2 Current Chemistry Ltd ISSN 1359-0294

Abbreviations
BAM Brewster angle microscopy
DMPE dimyristoyl phosphatidylethanolamine
DPPC dipalmitoyl phosphatidylcholine
RDS respiratory distress syndrome

Introduction
In 1929, von Neergaard [1] observed the difference in recoil forces between fluid- and air-filled lungs and was the first to attribute this difference to the influence of surface tension. It wasn't until the mid-1950s, however, that Partie [2] and Clements [3] demonstrated the existence of surface active agents in lung washings. Further research in this area was stimulated in 1959 when Avery and Mead [4] showed that a deficiency of lung surfactant was the primary cause of respiratory distress syndrome (RDS), which was the leading contributor to infant mortality in industrialized nations at the time [3]. While great strides have been made in reducing infant mortality due to RDS since that time, it remains the main impetus for current research on lung surfactant: up to 50,000 newborns are presently afflicted with RDS each year in the United States alone [6]. Furthermore, the processes involved in an adult form of RDS are not yet fully understood and mortality rates are 50% or more [7].

Lung surfactant is a mixture of approximately 90% protein and 10% lipid; about 35% of the lipid component is dipalmitoyl phosphatidylcholine (DPPC), which is the primary surface active element [8]. In addition, there are at least three main surfactant-specific proteins denoted as SP-A, SP-B, and SP-C [9].

The main physiological role of lung surfactant is to reduce the surface tension of the air—water interface in the alveoli, thereby providing two main benefits: first, the amount of energy required to inflate the lungs is reduced by increasing pulmonary compliance; second, the likelihood of lung collapse is reduced by decreasing elastic recoil, as well as the antisticking activities of lung surfactant. Recent reviews [10,11] summarize other roles of lung surfactant such as airway stabilization and antibacterial activity.

While in vitro measurements of the surface activity of lung surfactant have been taken for almost half a century, it is only within the past few years that methodologies that are free of experimental artifacts have been developed. This review will summarize the drawbacks of the classical methods and will describe how recent techniques overcome these difficulties. In addition, it will describe a method for direct visualization of the interface that may provide future insight into the function of lung surfactant.

In vitro measurement of surface tension-area isotherms of lung surfactant

One of the most commonly used tools for investigating the surface tension—area response of lung surfactant is the familiar Langmuir—Wilhelmy surface balance (as first used by Clements [3] in his pioneering work on lung surfactant). There are, however, numerous drawbacks to this apparatus. Because of its large size, the Langmuir—Wilhelmy surface balance requires large sample volumes and is difficult to isolate from the environment with regards to temperature and impurities. Furthermore, the adherence of proteins to the plate could alter the contact angle and lead to incorrect surface tensions [12,13]. Finally, the classical Langmuir—Wilhelmy surface balance cannot be cycled dynamically since the barrier would create waves in the trough [13].

To overcome these difficulties, the pulsating bubble surfactometer [14] was developed. This consists of a chamber filled with the sample and a small air bubble which is formed at the end of a plastic capillary. The surface area of the bubble is varied between two fixed values by changing the pressure in the chamber; the resulting surface tension is computed from the Laplace equation of capillarity for spherical bubbles. The assumption that the bubbles are spherical introduces error into the calculated surface tension which may become particularly important at low surface tension [15]. While a procedure exists to account for this effect [16], it is not generally applied. The effect of surface dilational viscosity is also generally ignored, yet this factor has the potential to give substantial errors in surface tension [15,16].

Further shortcomings of the pulsating bubble surfactometer restrict its range of applicability. Although it is possible to study adsorption kinetics and stability with the pulsating bubble surfactometer, it is not commonly
used for this purpose [17]. It is not possible to investigate the effect of varying the amount of compression, because the bubble size must be varied between two fixed values. Furthermore, it is not practical to explore the variation in surface tension with area since only the maximum and minimum areas are generally known. Finally, because it was designed to study soluble materials, it is not convenient to use the pulsating bubble surfactometer to study insoluble monolayers at the air-water interface.

In addition to these weaknesses, the Langmuir–Wilhelmy surface balance and the pulsating bubble surfactometer do not closely duplicate the major trends observed in in situ experiments of the surface tension of the alveolar surface. First, low surface tensions generated in situ were obtained in a quasistatic manner [18–23]. In comparison, low surface tensions are only attained with the pulsating bubble surfactometer under high cycling rates i.e. 20 cycles per min [24]. Second, the film must be compressed by 50–80% with the Langmuir–Wilhelmy surface balance and pulsating bubble surfactometer to attain near-zero surface tension [25], this is almost three times greater than the in situ measurements [22]. Third, the Langmuir–Wilhelmy surface balance and pulsating bubble surfactometer cannot reproduce the in situ stability of the compressed film [12,25]. For example, in a recent investigation with the pulsating bubble surfactometer, the film surface tension increased from near-zero to 10 mN m⁻¹ in about three minutes (as determined from pressure data) [26]. A similar in situ increase in surface tension required from at least 30 minutes [18] up to 70 minutes [20]. Fourth, in situ cycling of the lung lining film produces considerably less hysteresis than predictions based on studies conducted with surface balances [25]. Finally, although in situ surface tensions are very insensitive to temperature [21], a recent study conducted with the pulsating bubble surfactometer showed a significant effect of temperature [27].

The most likely explanation for the above discrepancies is that the Langmuir–Wilhelmy surface balance and the pulsating bubble surfactometer suffer from the problem of film leakage [13,24,25]. Film leakage is a problem that has been ignored by most investigators and can lead to the type of patterns which have been interpreted erroneously as being intrinsic properties of the lung surfactant [12]. Leakage occurs because of a fundamental thermodynamic principle: at a sufficiently low surface tension, the surface active molecules can spread from the water/air interface onto the surrounding solid; this process will decrease the free energy of the system.

Film leakage has been demonstrated to occur in the Langmuir–Wilhelmy surface balance by using 3H- and 14C-labelled DPPC to show that 14% of the film coated the walls of the trough [28]. It was also directly observed in the pulsating bubble surfactometer by viewing the capillary supporting the bubble under high magnification [29]. Moreover, while a modification of the standard pulsating bubble surfactometer methodology (designed to minimize film leakage) improved its ability to generate low surface tensions, it still could not do so in under ten cycles for a synthetic lipid–surfactant mixture [29].

The only way to avoid the possibility of film leakage is to eliminate the potential pathways for leakage to occur. This can be accomplished by using a captive bubble geometry, in which a bubble of air is floated to the top of a chamber full of lung surfactant, thereby eliminating the need for barriers or capillaries. Such a device, called the captive bubble surfactometer, has been built by Schurz and his coworkers [22] and has recently been improved [30]. There is no need to pierce the interface in order to measure the surface tension of the bubble; surface tension, area, and volume are determined by measuring the height and diameter of the bubble [31]. By eliminating the possibility of film leakage in this manner, the captive bubble surfactometer is able to reproduce all of the trends observed in in situ experiments [24,25,32].

In addition to overcoming the difficulties associated with film leakage, the use of the captive bubble surfactometer has recently been used to conduct several investigations that would be difficult, if not impossible, to achieve with the Langmuir–Wilhelmy surface balance or pulsating bubble surfactometer. For example, it has been demonstrated that it is possible to obtain a nearly hysteresis-free surface tension-area isotherm by avoiding over-compression of the interface [25,32]. The film leakage effects in the Langmuir–Wilhelmy surface balance and the pulsating bubble surfactometer make precise control over the compression ratio impossible. In fact, film leakage onto the plastic capillary in the pulsating bubble surfactometer results in an effective area compression of about 13% rather than 50% based on the change in bubble radius [29].

A phenomenon called 'bubble clicking' has been reported, in which a bubble with a low surface tension in the captive bubble surfactometer spontaneously increases its surface tension and decreases its area. The rejection of non-DPPC components from the interface is thought to be associated with this process, leading to an interface that is highly enriched in DPPC [24,25]. Conversely, a DPPC-enriched film may be formed on a fresh interface by 'adsorption clicks' which imply the sudden cooperative movement of a large number of molecules into the interface [32].

Different subfractions of rabbit lung surfactant were also investigated with the captive bubble surfactometer. It was possible to differentiate among the subfractions and to conclude which ones determine the alveolar surface tension in a normal lung [33].

Evidence for a 'surface-associated reservoir' was obtained by depleting the bulk phase surfactant in the captive bubble chamber. SP-A promoted the movement of surface
active material from the reservoir to the interface. These findings are supported by evidence for the existence of such a reservoir [34].

Finally, the captive bubble surfactometer has been used to explore the role of SP-A in lung surfactant [35]. Among other roles, it was found that SP-A enhances the rate of adsorption, reduces the incidence of bubble sticks, and reduces the amount of compression required to obtain a near-zero surface tension. It is doubtful that similar observations could be made with the Langmuir-Wilhelmy surface balance or the pulsating bubble surfactometer because of their previously described shortcomings.

Despite the fact that the captive bubble surfactometer is a substantial improvement over the Langmuir-Wilhelmy surface balance and the pulsating bubble surfactometer, there is still the need for further improvement. An improved method of calculating the surface tension of captive bubbles has been developed that allows multiple measurements on a single bubble [36]. The use of this method would provide an indication of the quality of the surface tension measurements [36]. Also, the analysis of the data is time consuming and complicated, while neither bubble formation nor bubble cycling are automated [29]. Future automation of these procedures would enhance ease-of-use and speed up data acquisition.

Surface pressure-molecular area isotherms of DPPC monolayers

A second development in the measurement of the surface activity of lung surfactant concerns the measurement of the DPPC surface pressure-molecular area isotherm. It has been recently shown that a pendant drop technique, axisymmetric drop shape analysis, can be used in a manner analogous to that of a classical Langmuir-Wilhelmy surface balance to study insoluble monolayers at the air-water interface [37]. The advantages of axisymmetric drop shape analysis over the Langmuir-Wilhelmy surface balance include a high degree of automation, a small sample size, and the ease with which the sample can be isolated from the environment. Moreover, since axisymmetric drop shape analysis does not suffer a similar restriction in compression rates as the Langmuir-Wilhelmy surface balance, a study was conducted to examine the effect of compression rate on the shape of the DPPC surface pressure-molecular area isotherm. The results indicated that the rate of compression of the monolayer had no effect on the shape of the DPPC isotherm [38] and were interpreted by considering the structure of the interface (see below).

A second investigation of DPPC monolayers with axisymmetric drop shape analysis [39] focused on the liquid-expanded/liquid-condensed phase transition in the surface pressure-molecular area isotherm. The nature of this phase transition is controversial and is the subject of much debate [40]. It was found that a physiologically realistic method of forming the monolayer results in a very different type of phase transition than the method used in an unnatural "ideal" technique [39].

Interfacial visualization by Brewster angle microscopy

Until now, surface tension is generally the only interfacial property of lung surfactant that has been investigated. All information about the structure of the interface has been inferred from plots of surface tension versus time or surface area. Future insights into lung surfactant function, however, may be obtained by direct visualization of the interfacial morphology with a technique called Brewster angle microscopy (BAM) [41]. In such an experiment, a p-polarized light source illuminates the interface at the Brewster angle of the bulk phase. Material at the interface leads to a measurable change in the reflectivity, allowing direct visualization of the interfacial morphology with a spatial resolution of 4 μm. The direct visualization of the interface of a monolayer during compression provides physical justification for the different regions on the surface pressure-surface area isotherm [41]. This visualization technique has been used to compare the interfacial morphology of monolayers of DPPC and dimyristoyl phosphatidylethanolamine (DMPE) [42].

These experiments demonstrate that dissimilarities in morphology lead to different phase behaviours: DMPE shows an additional phase transition that does not occur in a DPPC monolayer because the large head group of DPPC prevents the complete ejection of chains. In addition, differences in the collapse behaviour of the two monolayers can be directly visualized by BAM [42].

Evidence obtained with the BAM technique has also recently been used to explain the rate dependence of the collapse pressure and limiting molecular area of a monolayer of octadecanol [43]. As the amount of octadecanol spread onto the surface is increased the proportion of condensed phase structures increases, which in turn results in more defects and irregularities in the monolayer. Upon compression, this less regular structure is preserved, leading to a shift of the surface pressure-molecular area isotherm to the right and to an earlier collapse. In comparison, one explanation for the fact that there is no similar rate dependence for a DPPC monolayer is that BAM experiments show that it is always spread as a homogeneous phase [44].

In the future, BAM may also be used to provide insight into the function of lung surfactant. Currently, the composition of the interface can only be inferred by comparing the surface compressibility of lung surfactant with that of DPPC monolayers [24,52]. By direct visualization, it may be possible to quantify the amount of DPPC in the interface under various conditions.
Conclusions
Recent advances in the area concerning lung surfactant can be summarized in two points. First, the classical methods of measuring the surface tension of lung surfactant and DPPC monolayers are now known to be inadequate for this purpose and two recently developed techniques, the captive bubble surfactometer and axisymmetric drop shape analysis, overcome the drawbacks of the classical methods.

Second, by using BAM to visualize the interface directly, characteristics of the surface pressure-molecular area isotherms of DPPC and other insoluble monolayers can be explained. Application of BAM to lung surfactant may provide further insight into the function of lung surfactant in the future.

Acknowledgements
It is with deep regret that we note the sudden and untimely death of Robert M. Pirkop, with days after completion of the final draft of this article.

This work was funded by the Medical Research Council of Canada (MT-14362).

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Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
** of outstanding interest


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Support for the existence of a reservoir containing excess surface active material is introduced. SP A promotes the movement of material from the reservoir to the interface, while serum proteins inhibit this process. In vivo evidence for the existence of such a reservoir is also presented.


A pendant drop technique is used to measure rate dependence of the surface pressure-molecular area isotherm of a DPPC monolayer. It would not be possible to undertake a similar experiment with a conventional surface balance. The shape of the isotherm is insensitive to the rate of compression due to the molecular structure and the molecular pattern upon formation of the film.


BAM is used to visualize directly the interface for two phospholipid monolayers. Differences in domain structure are described which lead to different phase behaviours and collapse.


Support for the existence of a reservoir containing excess surface active material is introduced. SP-A promotes the movement of material from the reservoir to the interface, while serum proteins inhibit this process. In vivo evidence for the existence of such a reservoir is also presented.


A pendant drop technique is used to measure rate dependence of the surface pressure-molecular area isotherm of a DPPC monolayer. It would not be possible to undertake a similar experiment with a conventional surface balance. The shape of the isotherm is insensitive to the rate of compression due to the molecular structure and the molecular pattern upon formation of the film.


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Behavior of proteins at interfaces
John L Brash

The strong tendency of proteins to accumulate at interfaces is significant in many scientific and technological fields. Recent efforts to understand protein interfacial behavior include the use of engineered mutants, imaging with atomic force microscopy, and calorimetry. Advances have been made in designing solid surfaces that are protein repellent.

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Current Opinion in Colloid & Interface Science 1996, 1 582–588
Current Chemistry Ltd ISSN 1359-0294

Abbreviations
AFM atomic force microscopy
BSA bovine serum albumin
CD circular dichroism
dSC differential scanning calorimetry
ESR electron spin resonance
HK high molecular weight kininogen
PEO polyethylene oxide
PMMA poly methyl methacrylate
vWF von Willebrand factor

Introduction
Proteins are macromolecular amphiphiles and as such are endowed with strong surface activity. As a consequence they have a natural tendency to migrate to and accumulate at the interfaces between phases. The behavior of proteins at interfaces is of importance in two ways. First it is of intrinsic scientific interest and has the potential to reveal information about protein structure and function. Second it is of enormous practical and technological importance. Protein adsorption plays a key role in the formation of foams in protein solutions, in the separation of proteins in mixtures by chromatography and electrophoresis, in the response of the body to implants such as heart valves, joint prostheses and contact lenses, in the functioning of biosensors, in immunosorbent assays, and in the fouling of surfaces in bioprocessing operations. As a result there has been considerable research activity directed towards the understanding of proteins at interfaces. Comprehensive sources which should be consulted for background include references [1–5]. The present discussion is focused on the most recent trends and is organized accordingly.

Use of mutants in mechanistic studies
A major recent development has been the appearance of a number of papers dealing with sets of protein variants differing in amino acid composition in a defined manner [6–11]. This approach has the potential to elucidate adsorption mechanisms. Using mutants of bacteriophage T4 lysozyme, McGuire et al. [10–11] showed that substitution of the isoleucine at position three, for example, with cysteine and tryptophan, changed the stability of the protein and that adsorption to silica surfaces reflected these changes. They also showed [6–7], using in situ circular dichroism (CD) methods [11], that the less stable mutants also suffered a greater loss of α-helix upon adsorption. Arnold and coworkers [8–12] studied the adsorption of cytochrome c variants, which differed in the number of surface histidines, to solid substrates (metal affinity chromatography supports) having varying densities of copper ions. They showed that protein–substrate binding energy increased with both histidine and copper content and that adsorption data fitted well to the Temkin isotherm which requires a uniform distribution of adsorption site energies. It was argued that this is to be expected if the protein adsorbs ‘multivalently’ to a substrate having a dense and random distribution of binding sites.

Another application of protein variants is the introduction of chemical functions that can be used to immobilize a protein to a solid substrate. Jiang et al. [7] have studied a sperm whale myoglobin variant in which alanine 126 is replaced by cysteine, thus providing a surface thiol that binds the protein to a substrate-localized silane. They have shown that the protein retains much of its structure and function in the immobilized state.

Structure and conformation of proteins at interfaces
Scanning probe microscopy methods
Direct imaging methods based on scanning probe microscopies are finding increasing use in studies of adsorbed proteins. Atomic force microscopy (AFM) in particular is being used to good effect [13–15,16–21,22]. Early work was hampered by the tendency during scanning to rearrange rather than image the adsorbed protein due to the relatively high lateral force exerted by the tip. Limitations on resolution in AFM imaging due to the finite tip radius have also been encountered [20]. The rearrangement effect can be minimized by operating in tapping mode in which the tip oscillates perpendicular to the surface as it scans [14]. Images of adsorbed lysozyme and F-actin filaments in buffer have been obtained by this approach [14–21]. A series of studies of von Willebrand factor (vWF), a very large multimeric protein (5 x 10^3 to 2 x 10^7 Da) involved in hemostasis and thrombosis through its role in platelet adhesion, has been carried out by Marchant et al. [20,22]. A flow-induced conformational change is seen to occur at shear conditions which correspond to the onset of shear-dependent platelet adhesion to vascular subendothelium, mediated by vWF.
Ph.D. Related

Publication #4
An investigation of the compression rate dependence on the surface pressure–surface area isotherm for a dipalmitoyl phosphatidylcholine monolayer at the air–water interface

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An investigation of the compression rate dependence on the surface pressure-surface area isotherm for a dipalmitoyl phosphatidylcholine monolayer at the air water interface

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Received 27th November 1995; accepted 1st February 1996

Abstract

In this study, the effects of the monolayer compression rate on the shape of the surface pressure-surface area (π-A) isotherm were investigated for dipalmitoyl phosphatidylcholine (DPPC) at the air water interface. Both axisymmetric drop shape analysis and the Langmuir-Blodgett film balances were used for this study. It has recently been shown that a higher rate of compression shifts the lower part of the π-A isotherm to the right. However, the results of this DPPC study show no significant effect on the π-A isotherm shape for the range of compression rates used [1-30°/min] per molecule per minute. The varied influence of the compression rates on different monolayers is explained by considering the molecular structure and the molecular nature of the domains, as well as the molecular pattern upon monolayer formation.

Keywords: Air waterinterface, Axisymmetric drop shape analysis, Compression rate, Dipalmitoyl phosphatidylcholine, Monolayer, Surface pressure-surface area isotherm

1. Introduction

Compression of an insoluble monolayer at the air water interface yields data enabling one to plot a surface pressure-surface area (π-A) isotherm. These plots, in turn, provide information about the surface properties of the insoluble material. The shape of the isotherm is crucial in obtaining a proper understanding as to what is occurring at the molecular level. For example, the Gibbs elasticity of a monolayer is determined from the slope of the π-A isotherm.

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It is known that the shape of the π-A isotherm is dependent on many conditions including temperature [1-4], impurities [5], and method of monolayer formation [6]. Recently, it has been shown [7] that the rate of monolayer compression has an effect on the shape of the π-A isotherm for an octadecanol monolayer. To the authors' knowledge, this was the first formal attempt at describing the effects of the rate of compression on the π-A isotherm. In their study, Kwok et al. [7] showed that increasing the compression rate shifts the lower part of the π-A isotherm to the right. Using Brewster angle microscopy (BAM) as a supporting technique, they argued that if more time is allowed
for the condensed-phase regions of "islands" to coalesce and rearrange to a slower compression, the compressibility of the monolayer is reduced [7]. Hence, a faster compression would result in higher compressibility and the lower part of the isotherm would be shifted to the right in the manner shown in Fig. 1. This change is significant: for example, using data from Kwok et al. [7], at a surface pressure of 10 mJ m⁻², the area per molecule changed by approximately 30% between the fastest and slowest compression rates.

In this study, the influence of the rate of compression is examined for a dipalmitoyl phosphatidylcholine (DPPC) monolayer at the air-water interface. DPPC π-4 isotherms have been investigated extensively due to this material's biological significance. For example, DPPC is the major component of pulmonary surfactant [1.8].

Although the situation in the lungs is clearly a dynamic one, most workers investigating the π-4 isotherm for DPPC have done so quasi-statically. This is understandable considering the techniques used in the past, such as a Langmuir trough [1.5.8]. Hence, it would be of interest to see how the surface properties of DPPC are affected by the rate at which the monolayer is compressed. This is a valid concern since, if the shape of the isotherm is altered due to the changing of the compression rate, the validity of previous investigations in relation to the dynamic situation in the alveoli would have to be questioned. Also, it would be advantageous to have DPPC data for various compression rates in order to make comparisons with in vitro lung surfactant experiments performed under dynamic conditions.

Only a few studies which mention, in passing, the compression rate effects on DPPC π-4 isotherms [9-11] were found. For example, Nag et al. [9] noted that the DPPC π-4 isotherms maintained the same approximate shape for two different rates of compression: 7 and 240 Å² per molecule per minute. Vilaillonga [10] stated that the "general features" of the DPPC π-4 isotherm were similar for all three compression rates used (4, 8 and 12 Å² per molecule per minute). Phillips and Chapman [11] suggested that the "exact" shape of the isotherm depends on the rate of compression. These studies, however, did not include any plots to compare the isotherms for the different compression rates. Hifeda and Rayfield [5], in their efforts to demonstrate the so-called liquid-expanded liquid-condensed phase transition is a first-order transition, concluded that high compression rates result in a change in the π-4 isotherm. The range of compression rates used by Hifeda and Rayfield was 0.1-2 Å² per molecule per minute, and the effect that they showed for pentadecanoic acid [5] is not as significant as the change for octadecanol demonstrated by Kwok et al. [7].

The range of compression rates used in our study of DPPC was broad (1.60-371 Å² per molecule per minute) and, in addition, many intermediate rates were used. Also, two surfactant balance techniques were employed, namely axisymmetric drop shape analysis and the Langmuir-Wilhelmy film balance.

2. Materials and methods

Three samples of dipalmitoyl phosphatidylcholine (DPPC), two of which were approximately 99% pure and one was 99 - % purity, were obtained from Sigma and used without further purification. The spreading agent was chloroform, obtained from Aldrich and Caledon. The water used for all experiments was demineralized and doubly distilled.

Axisymmetric drop shape analysis (ADSA)
determines the surface tensions and contact angles of pendant and sessile drops by applying the Laplace equation of capillarity to the drop profile [12-14]. ADSA as a film balance has been discussed in detail before [15], and will be reviewed here only briefly. A pendant drop of pure water is formed at the end of a silver capillary in a quartz cuvette which is placed within a temperature control cell. This isolation protects the drop from contamination and air currents, in addition to providing excellent environmental control and temperature. All ADSA experiments were performed at 23°C.

Using a micromanipulator and a microsyringe, a known amount of a DPPC chloroform solution of known concentration is deposited onto the drop. Then, by removing water from the bulk phase of the drop, and hence reducing the surface area, compression of the monolayer is achieved. Although the surface area is changed indirectly by controlling the volume, this change is linear for the small volumes used in the pendant drops [15].

Images are acquired throughout the compression process and are stored in a Sun SPARCstation 10. ADSA then analyzes these images to calculate the surface tension of the drop surface area and the drop volume. The surface pressure is simply defined as the surface tension of pure water minus the surface tension of the monolayer-coated pendant drop. The area per molecule value is calculated easily since the number of molecules deposited onto the drop and the surface area of the drop are both known. With this information, the π-α isotherm can be plotted.

The rate of compression of the monolayer can be controlled in two ways [11]: (1) by varying the amount of surfactant deposited on the drop, and (2) by changing the rate at which pure water is removed from the bulk phase of the pendant drop (by varying the speed with which a motor moves the plungers of the syringe which is connected to the capillary [11].

\[
\text{Area} = \frac{1}{\text{Molecule}} \times \text{Molecule} \times \text{Minutes} \times \text{Minute} + 1
\]

Hence, the "compression rate" refers to the left-hand side of Eq. 11, i.e., area per molecule per minute and "rate of area change" refers to the second term on the right-hand side of the same equation (area per minute). Also, each experimental run had an initial drop surface area of 15.50 ± 0.004 cm²; the ± values are the 95% confidence limits. Therefore, the different amounts of surfactant deposited on to the drop correspond to different initial surface concentrations (measured in molecules per Angstrom squared).

For the ADSA experiments, a total of 17 compression rates was used. Sixteen of these were obtained using four rate of area change settings and four different amounts of DPPC deposited on to the drop, corresponding to a range of 1.60 - 3.71 A² per molecule per minute. The 17th compression rate was obtained by using an additional rate of area change (5 × 10⁻¹² cm² min⁻¹) with 5 × 10¹⁲ molecules deposited onto the drop, corresponding to a compression rate of 1.60 A² per molecule per minute. This was done to further broaden the range. Table I summarizes the various rates of area change and number of molecules deposited, for all the ADSA compression rates used. The slower compression rates are similar to those used by other workers [5, 10, 16]. The upper limit of the range was restricted by the limitations of our image acquisition system (currently a maximum of 25 images per second). At least three runs were repeated for each of the compression rates used.

Langmuir-Wilhelmy balance (LB) measurements (LAUDA film balance) were performed under similar experimental conditions. The compression rates are expressed as a function of the number of molecules deposited onto the drop and the rate of area change.

Table I
The 17 ADSA compression rates as a function of the number of molecules deposited onto the drop and the rate of area change.

<table>
<thead>
<tr>
<th>Rate of area change</th>
<th>Number of molecules deposited onto the drop</th>
</tr>
</thead>
<tbody>
<tr>
<td>cm² min⁻¹</td>
<td>3 × 10⁸</td>
</tr>
<tr>
<td>1 × 10¹⁰</td>
<td>222</td>
</tr>
<tr>
<td>2.5 × 10¹⁰</td>
<td>106</td>
</tr>
<tr>
<td>4.0 × 10¹⁰</td>
<td>48</td>
</tr>
</tbody>
</table>

* A² per molecule per minute
* Initial surface area: 15.50 ± 0.004 cm² for all pendant drops.
pression rates used for the trough experiments were: 3.80, 5.00, 11.9, 25.8 and 31.8 Å² per molecule per minute. The temperature for these experiments was maintained at 20°C.

3. Results

The π-4 isotherms obtained with ADSA are shown in Figs. 2-4. Due to the minute amounts of surfactant used in ADSA experiments and the associated uncertainties, slight shifts are noticeable in the π-4 isotherms. It is for this reason that the 95% confidence limits for the area per molecule values are included.

Fig. 2 shows the effect of varying the rate of area change as the number of molecules is held constant. However, since the initial area was the same for all compressions, this set of experiments is equivalent to fixing the initial surface concentration of DPPC. Fig. 3 shows the effect of changing the initial surface concentration as the rate of area change is used. Fig. 4 shows a comparison between the slowest (1.60 Å² per molecule per minute) and the fastest (371 Å² per molecule per minute) ADSA compression rates. Since the lateral shifts are all within the 95% confidence limits, we can conclude that they are a result of the uncertainties in the amount of DPPC and are not a result of compression rate effects. Also, the shifted DPPC isotherms are parallel to one another throughout the compression. In the case of octadecanol [5], the compression rate effect changed the isotherm shape only at low surface pressures. Therefore, it is clear from these three plots that the rate of compression has no effect on the shape of the isotherm. Regardless of how the compression rate is changed (i.e. by varying the number of molecules deposited onto the drop or by varying the rate of area change), the isotherm maintains the same shape throughout the entire range of compression rates used.

The π-4 isotherms for the LB measurements are shown in Fig. 5. For these experiments, the initial surface concentration of DPPC was held constant while the speed was varied. Here, too, the isotherm shape is maintained throughout the range of speeds used. Hence, regardless of whether ADSA or LB was used, the shape of the isotherm is consistent for all

![Graph showing ADSA π-4 isotherm for DPPC at 23°C](image)

Fig. 2. ADSA π-4 isotherm for DPPC at 23°C: effect of the compression rate as the initial surface concentration is held constant and the rate of area change is varied. The 95% confidence limits for area per molecule values are included.
Fig. 3. ADSA - Isotherm for DPPC at 23 C. Effect of the compression rate as the rate of area change is constant and the initial surface concentration is varied. The ±5% confidence limits for area per molecule values are included.

Fig. 4. ADSA - Isotherm for DPPC at 23 C. Effect of the compression rate. The two rates shown are the fastest and slowest ADSA compression rates used in this study. It is apparent that there is no compression rate dependence. The ±5% confidence limits for area per molecule values are included.
compression rates. However, it should be noted that the shapes of the ADSA isotherms and the LB isotherms are not identical. The LB isotherms show a distinct plateau at approximately 80 A² per molecule which is absent in the ADSA isotherms. This issue has recently been addressed in detail [6]. In essence, Jyoti et al. [6] argued that the manifestation of the so-called liquid-expanded liquid-condensed phase transition for DPPC depends on the different methods of monolayer formation used in each type of experiment.

4. Discussion

The issue that must be addressed here is the influence of the compression rate on different types of insoluble monolayers. As stated in the Introduction, Kwok et al. [7] investigated another monolayer type, octadecanol, and found that increasing the rate of compression shifted the lower part of the isotherm to the right, i.e. increasing compressibility for faster compression. It should be noted that the range of compression rates used in this study is about 10 times greater than that in the earlier octadecanol study, and yet no effect is noticeable.

DPPC has two aliphatic chains with a large choline head group, compared to the monolayer construction of molecules with a single alkyl chain and a small -OH head group. Hence, the number of deposited DPPC molecules on the same drop surface area would be much less than that of the small-size single-chain alcohol. The number of octadecanol molecules deposited onto the drop surface ranged from approximately 5.0 x 10¹³ to 1.5 x 10¹⁴ molecules ["]. Using the same criteria in this study, the corresponding range was only 3.4 x 10¹¹ to 5.0 x 10¹³ DPPC molecules. This range is over 90% smaller than that used in the case of octadecanol ["]. Therefore, we see that relatively few DPPC molecules can be deposited on the surface and that the upper limit of the amount of DPPC is equal to the lower limit used in the octadecanol study ["]. Since there are fewer molecules at the interface for DPPC, their realignment during compression may not be as complicated.

The initial state of a monolayer is a crucial condition for its thermodynamic and relaxation behaviour. The state of a monolayer depends strongly on the phase properties of the amphiphilic monolayers. There is a large difference between the initial conditions of single alcohol monolayers
and DPPC monolayers. Results of the BAM study of octadecanol monolayers have shown that under the usual spreading conditions, irregularly curved, disordered mixtures of condensed-phase structures and gaseous phases exist [17]. These depend mainly on the spreading conditions and the initial area per molecule. Upon compression, regular condensed-phase structures cannot be formed but rather an irregular morphology is preserved. A more condensed phase presents more defects and irregularities. Thus, compression of the inhomogeneous monolayer of octadecanol shows a strong rate dependence as expected. On the other hand, DPPC is spread in a single fluid phase at low density [17,18]. The monolayer material is distributed homogeneously and differences in the initial conditions do not exist. Upon compression, regular condensed-phase structures of DPPC are formed. Thus, the compression of a homogeneous gas-like DPPC monolayer does not produce a compression rate effect on the $\pi-A$ isotherms.

Upon compression, a DPPC monolayer forms flexible domains in the coexistence region of low density and condensed phases and can change the domain shapes quickly [19]. For instance, the domains in DPPC monolayers form triskelions at very low compression rates but evolve more arms at intermediate compression rates and are branched at high compression rates [20]. Nag et al. [9] showed that increasing the rate of compression decreased the size of the DPPC domains but had no influence on the isotherm shape. Hence, even though the shape and size of the domains are compression rate dependent, the spread molecules rearranged themselves to fit in whatever space was available.

The capacity of DPPC to adjust to an area reduction may be attributed to the change in orientation of the hydrophobic tails of the DPPC molecule. Many studies [21-23] have focused on the tilt angle (from the surface normal) for the DPPC tails. In their pioneering study, Tardieu et al. [21] showed for DPPC that when the average area per molecule per polar group decreased, the tilt angle also decreased (i.e., the orientation became more vertical). However, the large head group prevents the complete ejection of the chains so that a continuous decrease of the tilt angle upon compression occurs. Not all interaction data have revealed that the tilt angle of DPPC tails remains prior to film collapse [24]. This means that the chains can rotate freely around their long axes and form a rotator phase with a cross-section near $20 \AA^2$ per molecule in the highly compressed state [25]. However, this hindered rotator phase does not affect the $\pi-A$ isotherm shape when the compression rate is varied. Single-chain compounds, like octadecanol for example, are amenable to the densest packing of the long hydrocarbon tails. There are various crystalline packings of the chains on alkane crystals with a chain cross-section below $18.6 \AA^2$ per molecule [26].

It is interesting to note that the range of area per molecule over which the compression occurs is broader for DPPC than for octadecanol. It would seem that since the DPPC molecule is much larger than an octadecanol molecule, the exertion of a surface pressure is detected at a higher area per molecule value. However, because of the malleable properties of the domains, the DPPC monolayer may be compressed over a larger area per molecule range.

It is known that this ordering of the DPPC tails towards the vertical requires energy [21,23]. This may be an indication as to why the range of compression is so much broader for DPPC than, for example, octadecanol [27]. Buontempo and Rice [27] used infrared external reflection spectroscopy to study an octadecanol monolayer at the air water interface. As the surface pressure of the monolayer increased, they found that although the intramolecular order of the octadecanol chain increased, the tilt angle remained invariant [27]. If the tilt angle of the octadecanol tail was invariant, the monolayer would not be as malleable and hence, the range of area per molecule over which a compression occurs would be correspondingly small. Furthermore, this characteristic of an octadecanol molecule would be reflected in the shape of the $\pi-A$ isotherm as the compression rate was varied. In the case of DPPC, however, as the monolayer is compressed, energy is used to make the chains more vertical, extending the range of area per molecule over which the compression occurs.
5. Conclusions

It was found that the rate of compression had no effect on the shape of the DPPC α-A isotherm. This is explained by considering the specific structure of the DPPC monolayer as well as the flexible nature of the domain shape. As the monolayer is compressed, the tails become more vertical and thus provide room for further compression. This process requires energy, extending the range of area per molecule over which the compression occurs. This property, in addition to the relatively few molecules that are in the monolayer, allow for a system which adjusts well to a reduction in area. The initial regular pattern of the molecules in the monolayer may also contribute to the compression rate independence of the monolayer.

Acknowledgements

We would like to acknowledge the financial support of the Medical Research Council of Canada (MT-2462), and postgraduate fellowships from Fonds pour la Formation de Chercheurs et l'Aide à la Recherche (A.J.) and the Natural Sciences and Engineering Research Council of Canada (R.M.P.)

References

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Publication #5
On the Manifestation of the Liquid-Expanded/Liquid-Condensed Phase Transition of a Dipalmitoyl Phosphatidylcholine Monolayer at the Air-Water Interface

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Key words: Dipalmitoyl Phosphatidylcholine: Phase Transition: Surface Tension: Monolayer: Surface Pressure Isotherm: Phospholipid
ABSTRACT

Using Axisymmetric Drop Shape Analysis as a film balance, the surface properties of a dipalmitoyl phosphatidylcholine monolayer at the air-water interface were investigated. In particular, the controversial liquid-expanded/liquid-condensed (LE/LC) phase transition of the surface pressure-surface area isotherm was examined. The results indicate that the manifestation of this transition is dependent on the method used to form the monolayer. A "surface deposition" method results in a high concentration of lipid at the interface, and compression of this type of monolayer produces the LE/LC transition. When the monolayer is formed using "bulk deposition", this transition does not occur. The proposed process of lipid arrangement for this type of monolayer is: gaseous phase (G), G (or LE) - LC- solid phase (SC), and SC. Hence, the LC phase is present throughout the middle region of the isotherm.
1.0 INTRODUCTION

Due to the biological significance of a dipalmitoyl phosphatidylcholine (DPPC) monolayer at the air-water interface, the surface properties of this material have been investigated by many researchers. For example, the study of lung surfactant in the mechanics of breathing [1-5], as well as the modelling of lipid bilayers and cell membranes [6,7] require an understanding of the surface properties of DPPC. One tool used to investigate some of these characteristics is the surface pressure-surface area (π-A) isotherm. In the past, many studies have focused on the so-called liquid-expanded/liquid-condensed (LE/LC) phase transition [8-18] found in most published DPPC π-A isotherms. To date, this region of the DPPC isotherm is very poorly understood [9] and has been the subject of controversy for many years [18,19].

The confusion surrounding the nature of this phase transition is evidenced by the lack of agreement regarding the actual experimental data [7]. Some researchers have found the LE/LC region to be perfectly horizontal and extending over a range of about 20 Å²/molecule [15,17], while others show sloped transitions [8,16,18-21]. In some studies, the presence of a "shoulder" or "kink" in the isotherm [1,12,22-24] indicates the location of the LE/LC phase change. In our literature search, only a few studies [2,4,25] with no visible LE/LC phase change in the DPPC π-A isotherms were found.

Different laboratories investigating the surface properties of DPPC have used a variety of experimental apparatuses and methods [7,25,26]. Differences among these investigations include surface-balance equipment and methodology, purity of materials, spreading agent, subphase, temperature and the rate of compression of the monolayer. It should be noted, however, that our search to find any trend relating the experimental procedure and the
manifestation of the LE/LC phase transition was in vain.

Previous investigators, however, seem to agree that the kink in the \( T-A \) isotherm indicates some sort of phase transition. Hence, many have placed high priority on determining the order of this phase change. Those maintaining the LE/LC transition to be first order point to the zero-sloped transition along with the surface potential [27], fluorescence imaging [28] and electron microscopy evidence [12], all of which indicates that two phases of the lipid monolayer co-exist in this region. Second order transitions have also been considered. For example, Baret et al. [11] suggested the kink is caused by a break of rotational symmetry of the film. This is similar to the second order transition first proposed by Dervichian [10]. Some researchers have also claimed this DPPC phase transition is \( 3/2 \) order [29], and even as high as third order [30].

In this study, we examine the effect of the method of monolayer formation on the manifestation of the LE/LC phase transition. We also consider the process of lipid arrangement as the monolayer is compressed.

2.0 MATERIALS AND METHODS

Three samples of dipalmitoyl phosphatidylcholine (DPPC) were purchased from Sigma Chemical Co. (St. Louis, MO) over a period of nine months. Two of these samples (Samples 1 and 2) were approximately 99 \% pure and one (Sample 3) was 99 + \% pure (as claimed by Sigma; the 99 + \% pure sample is a further crystallization purification of the approximately 99 \% pure sample). All samples were used without further purification. Chloroform, from Caledon (Georgetown, ON), was used as the spreading solvent for most experiments. The other spreading agent was a hexane/ethanol (9:1 v:v) mixture. The hexane was purchased from Mallinckrodt
The subphase for our experiments was de-mineralized, doubly distilled water.

2.1 Experimental Set-Up

Axisymmetric Drop Shape Analysis (ADSA) is a novel technique for measuring surface tension and contact angles [31-33]. The use of ADSA as a film balance has been discussed in detail elsewhere [34], but will be reviewed here briefly. A pendant drop is isolated in a quartz cuvette which, in turn, is placed within a temperature/pressure cell. This protects the drop from air currents and contamination, as well as providing excellent control over the environmental conditions (i.e., temperature). All experiments were performed at 23 °C. The entire set-up is mounted on a vibration-free table to minimize any external vibrations. A Cohu CCD monochrome camera is mounted on a Leitz Apozoom microscope. The camera acquires images of a pendant drop which are then stored in a Sun SPARCStation 10 workstation. The present rate of data acquisition is two and a half images per second. ADSA software extracts the drop profile from the digitized images and uses the Laplace equation of capillarity to calculate the surface tension of the pendant drop. Other output of ADSA includes radius of curvature, drop volume and drop surface area, the latter making this methodology ideally suited for film balance investigations.

The volume of the pendant drop is controlled by a syringe connected to a stepper motor. By moving the plunger of the syringe, and hence adjusting the volume, the surface area of the pendant drop is changed. Although the surface area is changed indirectly, this change is linear for the small changes in volume used in pendant drops [34].
2.2 Measurement Procedure

A quantity of pure DPPC, on the order of 6 mg, was precisely weighed on a Mettler H20 mechanical balance and then dissolved in 100 ml of chloroform. 10 ml of this stock solution was diluted 1:5 with chloroform. The dilution is necessary for two main reasons: to deposit the minute amounts of DPPC required onto the pendant drop, and at the same time, to minimize any errors in weighing. A new surfactant solution was prepared on each day of experiments.

A drop of pure water was formed at the end of a Teflon capillary which is connected to a Hamilton syringe. The plunger of this syringe is attached to a stepper motor. An image was acquired and analyzed, providing the surface tension of the pure water substrate, \( \gamma_w \).

Using a microsyringe and micro-manipulator, a known amount (typically 5 \( \mu l \)) of the diluted surfactant solution was deposited onto the pendant drop. This deposition was achieved in one of two ways. In the first method, which will be referred to as "bulk deposition", the surfactant solution was applied directly onto the drop; penetration of the microsyringe needle into the bulk of the pendant drop is thus inevitable (Figure 1a). Hence, with this method, DPPC will be deposited in the bulk phase in addition to the surface. In the alternate method called "surface deposition", the surfactant solution is deposited onto the Teflon needle directly above the pendant drop (Figure 1b). The solution then flows down over the drop surface and the surfactant coats the pendant drop completely, with no DPPC deposited directly into the bulk phase. With the exception of the monolayer deposition method, the protocol for both experiments was identical.

Although the spreading agent has been shown to evaporate within 20 seconds [34], five minutes after surfactant solution deposition were allowed to elapse to ensure complete evaporation (the first minute after deposition elapsed with the drop in the outside environment;
the drop was then lowered into the cuvette. The surface area was then decreased by removing volume from the drop with the motor-controlled syringe.

In a previous study [35], we examined the monolayer compression rate dependence of DPPC $\pi$-A isotherms, using a range of rates from 1.60 to 371 Å$^2$/molecule/minute. We found that the rate of compression has no effect on the shape of the DPPC $\pi$-A isotherm [35]. In this present study, intermediate compression rates were used to determine the influence of the type of spreading agent, the sample purity and the method of monolayer formation on the shape of the $\pi$-A isotherm. Images were acquired every 0.5 to 30 seconds, depending on the rate of monolayer compression.

Every acquired image was analyzed, providing a surface tension measurement, $\gamma$, as well as the surface area of the pendant drop. The surface pressure ($\pi$) is computed by calculating the difference between $\gamma_0$ and $\gamma$, i.e.,

$$
\pi = \gamma_0 - \gamma
$$

3.0 RESULTS

Figure 2 shows $\pi$-A data from three individual drops with monolayers formed by bulk deposition of Sample 2; these monolayers were compressed at a compression rate of 172 Å$^2$/molecule/minute. It should be noted that the slight shifts in the area/molecule values seen in these isotherms, are most likely due to the inevitable errors in handling minute amounts of surfactant required in ADSA film balance experiments [34]. It is obvious that no distinct LE/LC phase transition is noticeable in the bulk deposition isotherms. The ADSA results of this method
of monolayer deposition agree well with other independent studies [8,19-21,23] in the gaseous phase ( > 90 Å²/molecule) and in the solid phase ( < 50 Å²/molecule), but deviate in the middle region where the LE/LC phase change is said to occur.

Figure 3 shows that the type of spreading solvent used (i.e. chloroform or hexane/ethanol) had no influence on the isotherm shape (the monolayers were formed by bulk deposition of Sample 3). As shown in Figure 4, the possible minor differences in the purity of the three DPPC samples also had no influence on the π-A isotherm shape. The data for these isotherms were obtained using the bulk deposition method of monolayer formation.

Figure 5 shows π-A data from three individual drops with monolayers formed by surface deposition of Sample 3: the rate of compression of these monolayers was 53.3 Å²/molecule/minute. Figure 5 clearly shows that the LE/LC phase transition occurs when the surface deposition method is used to form the monolayer. In our experiments, the transition occurs at a surface pressure of approximately 7 mJ/m², consistent with other studies which find that the transition occurs between 5 and 12 mJ/m² [5,8,12,16,18,19-22], depending on, among other things, the temperature range which was used in those studies (20 - 25 °C).

Clearly, the curves for monolayers formed by the two types of methods (i.e., bulk deposition and surface deposition) are very different. The influence of monolayer formation method is apparent from the difference in isotherm shape, specifically the manifestation of the LE/LC phase transition.

4.0 DISCUSSION

In order to permit a direct comparison between the two methods of monolayer formation,
Figure 6 shows the average of the isotherms in Figures 2 and 5. To determine the source of the difference in these isotherm shapes, we began by considering ideas previously suggested in the literature.

First, it has been claimed that contamination may affect the manifestation of the LE/LC phase transition [17]. However, the three separate samples of DPPC used in our experiments yielded identically shaped isotherms (Figure 4). Moreover, the same samples of DPPC and spreading solvents were used for both bulk deposition and surface deposition experiments. Furthermore, contamination from the environment is less likely when using ADSA than for a Langmuir type of surface balance due to the size of the interface and the ease with which it is isolated from the environment. Hence, contamination of the surfactant or the spreading agent is not likely to be the cause of the difference in the shapes of the isotherms.

Second, it has been suggested that the rate of compression may affect the shape of the isotherm [17]. However, when the monolayer was formed by bulk deposition, the rate of compression also had no influence on isotherm shape even though the range of speeds was over two orders of magnitude [35].

Third, it has been claimed that the type of spreading agent used affects the exact shape of the DPPC π-А isotherm [23,25]. However, as explained above, when bulk deposition was used, no influence of spreading agent could be detected (Figure 3).

Hence, we see that the manifestation of the DPPC LE/LC phase transition is dependent on the method used to form the monolayer. In order to understand this difference, consider the different arrangement of DPPC molecules resulting from the surface deposition method, in comparison to the method of bulk deposition. As first proposed by Gershfeld [9], we suggest
that the lipid molecules at the interface are constrained to remain there, whereas those in the bulk are free to adsorb to the surface. We believe that this fundamental difference affects the manifestation of the LE/LC phase transition: a detailed discussion follows.

Since DPPC molecules are virtually insoluble in water they will readily adsorb to the surface. For example, Putz et al. [36], using rabbit pulmonary surfactant data and treating the interface as if it was composed entirely of pure DPPC, found that the adsorption rate constants were about 10,000 times greater than the desorption constants. Even if experimental circumstances, such as geometries of bulk and surface phases might modify the phenomena somewhat, desorption can always be expected to be much slower than adsorption.

Furthermore, in our pendant drop experiments, gravity will aid the transport of any chloroform (and hence DPPC) in the bulk towards the drop surface, enhancing the rate of adsorption. That is, since chloroform is denser than water, its use as a spreading agent will aid the transport of DPPC towards the interface. The geometry of conventional film balances is different to our pendant drops. In fact, Mingins and Owen [37] cautioned against using chloroform as a spreading agent in conventional film balances since, in those the systems, the chloroform would tend to move the DPPC away from the surface.

In contrast, the DPPC molecules already at the interface desorb into the bulk at a much slower rate [36]. Gershfield [9] showed that the surface concentration of films formed with spreading agents are consistently higher than for adsorbed films. He suggested an "activation energy" for dissolution constrains molecules in a spread monolayer to remain at the interface [9].

Hence, the strong adsorption and limited desorption of DPPC molecules can help explain the manifestation of the LE/LC phase transition when the monolayer is formed by surface
deposition. To understand this, consider again the resulting effects of a higher concentration of lipid present in a film formed by surface deposition compared to one formed by bulk deposition. These effects, seen on the π-A isotherms (Figure 6), may be explained in terms of micelle formation [9]. As a surface-deposition film is compressed, the "extra" lipid present in the film may form "extra" domains. On the other hand, in a compressed bulk-deposition film these extra domains would not exist on the surface (i.e., they would be present as micelles in the bulk phase). The presence of additional domains in the monolayer may affect domain interactions or their distribution, resulting in the manifestation of the LE/LC phase transition. Gersfeld [9] suggested that the absence of lipid in the bulk (due to the activation energy barrier) causes the apparent transition to appear in the isotherm. He demonstrated this by showing that the spreading agent affects the LE/LC phase transition in lipids which exhibit this transition at surface pressures above their equilibrium spreading pressure, \( \pi_{\text{eq}} \) [9]. (In a saturated solution, the equilibrium surface pressure which results from adsorption of lipid to the surface is defined as \( \pi_{\text{eq}} \).) At 25 °C, the \( \pi_{\text{eq}} \) of DPPC is about 0.1 mJ/m\(^2\) [7,22] and the transition occurs well above this value, at about 7 mJ/m\(^2\) (Figure 5).

In the same vein, Horn and Gersfeld [7] plotted π-A isotherms for solvent spread and crystal spread DPPC monolayers. For the former, the LE/LC plateau occurred at approximately 9 mJ/m\(^2\) and extended for about 10 Å\(^2\)/molecule [7]. In the case of the crystal spread monolayer, the transition was evidenced by a smaller kink, just 2 Å\(^2\)/molecule wide, and occurred at a higher surface pressure of 22 mJ/m\(^2\) [7]. It should be understood that the crystal spreading method of Horn and Gersfeld [7] and our bulk deposition method have obvious differences, and that this may explain the discrepancy of the results between the two studies. Also, as demonstrated by
their study [17], the LE/LC phase transition found in the literature has many different forms. This issue will be addressed in detail below.

In a study of temperature, humidity and pH effects on DPPC γ-A isotherms, Colacicco et al. [38] also used two methods of monolayer formation. Direct comparison of their results to ours is difficult due to the various environmental conditions, and in addition, many of their isotherms have a minimum surface pressure of only about 10 mN/m (i.e., a maximum surface tension of about 62 mN/m²). However, depending on whether the monolayer was formed by adsorption from the bulk or by spreading, they also found the isotherm shapes were altered [38].

As explained in the Introduction, the LE/LC transition takes many forms in the literature, some with evident plateaux. For our ADSA surface deposition experiments, we observed a shoulder but not a long flat transition region (Figure 5). If the monolayer is formed by surface deposition, the amount of time allowed for the DPPC to dissolve into the bulk may influence the LE/LC transition shape.

To illustrate this point, we can examine the study of Hifeda and Rayfield [17], who found a zero-sloped transition region extending for about 20 Å²/molecule, used almost 10³ times the amount of surfactant that was used in our experiments. The surface area of their Langmuir trough was over 10⁴ times larger than that of our pendant drop and their carefully controlled experiment took several hours to complete [17]. The dynamics of this system however are not clear. To our knowledge the absorption of DPPC into the bulk has not been formally addressed, but if we consider Fick's diffusion equation, diffusion time is proportional to the square of the size of the system. Therefore, we would expect the desorption process to be faster in a pendant drop than in a Langmuir balance. Hence, we suggest the amount of lipid in the bulk could affect
the details of the LE/LC transition seen in the $\pi$-$A$ isotherm.

It should be noted that for our ADSA results the isotherms for the two deposition methods line up with one another within experimental uncertainty everywhere but the disputed LE/LC transition region of the isotherm (Figure 6). The experimental uncertainty calculated from the shifts of the isotherms was calculated to be 4.6% based on the average of 95% confidence limits from sets of at least 3 runs for each experiment. Hence, more than 4.6% of the DPPC would have to be unaccounted for before we would suspect our molecular area to be erroneous. The area/molecule value could be shifted for one of three reasons: (1) uncertainty in the amount of lipid deposited onto the drop, (2) lipid remaining on the Teflon capillary, (3) lipid in the bulk phase instead of at the surface. We believe that the first reason is not a major concern since at least three runs were done for each experiment; any errors in quantity would be random and would cancel out upon averaging. The second reason is possible if leakage occurs when the monolayer is being compressed or if some lipid is "left-over" after deposition of surfactant onto the needle with the surface deposition method. We do not believe leakage to be a concern since a new pendant drop of pure water was formed after each compression and the water surface tension was consistently verified to be the correct value (72.4 mJ/m$^2$ at 23 °C) in accordance with the literature.

Hence, the amount of lipid which remains in the bulk can not be more than 4.6% when the bulk deposition method is used. This estimate is slightly higher than the finding of Goerke and Gonzales [39]. Using tracer techniques to detect DPPC leakage from their Langmuir-Wilhelmy surface balance, they found that 2% of the lipid was within the bulk phase. Goerke and Gonzales [39] did not specifically mention how the monolayer was formed in their
experiments, nor were any π-A isotherms included in the paper, but they did discuss the "small 'kink' seen on DPPC surface tension-area isotherms". Therefore, we suspect that a method similar to surface deposition was used in their experiments. Thus, the 3% value is less than we would expect had bulk deposition been used. Whatever the exact value, it is a seemingly minute amount of lipid which makes the difference in the middle region of the DPPC π-A isotherm.

We propose that if a highly ordered monolayer is to be studied, surface deposition may be a suitable method of film formation. However, in the case of studying models of biological relevance, we suggest that (for the reasons given below) the more appropriate method of forming the monolayer is by bulk deposition.

As discussed earlier, the surface deposition method results in a type of monolayer that has lipid molecules constrained at the interface. We suggest that in the lungs, the monolayer would be formed by adsorption of all but a minute amount (i.e. less than 4.6%) of the lipid to the surface. It is known that phosphatidylycerine is synthesized in type II alveolar cells, and then secreted into the alveolar hypophase [40]. (The adsorption process of DPPC from the bulk to the interface of this system is complicated by the presence of other surfactant lipids and proteins.) Also, Schürch and Bachofen [41] have recently described a "surfactant-associated reservoir". They suggest that the situation in vivo involves a surplus of surfactant near the interface that acts as a reservoir for the potential replenishment of the surface active film [41]. Hence, we believe that a film formed by surface deposition, with lipid constrained at the surface, would not model this phenomenon appropriately. We suggest that the bulk deposition method is more physiologically realistic than the surface deposition method, and thus, the rest of the discussion will be based on the results of the bulk deposition method alone.
The bulk deposition isotherms shown in Figures 2, 3 and 4 have three distinct phases: the first up to about 90 Å²/molecule, the second from 90 to about 50 Å²/molecule, and the third less than 50 Å²/molecule. As stated above, the first corresponds to the gaseous phase (G) and the third to the solid phase (SC). These are in agreement with other independent studies [8,19-21,23]; it is the description of the middle region of the isotherm which must undergo renewed consideration.

Denicourt et al. [18], proposed an overall transformation from the LE phase to SC phase and suggested that the LC phase acts as an interfacial phase between LE and SC. Hence, maintaining this logic, we suggest the middle phase of our ADSA π-A isotherms is a direct transition of gaseous phase (or LE) to a solid phase. The LC phase is inherent throughout the process, acting as a solidification front, but does not possess its own distinct phase transition. The three regions of the π-A isotherm and corresponding process of lipid arrangement would then be G, G (or LE)-LC-SC and finally SC. That is, in the middle region three types of lipid domains would exist simultaneously, with the SC phase slowly engulfing the encircling LC phase which, in turn, would merge into the surrounding G (or LE) phase. This would continue as the monolayer was compressed until eventually no G phase remained, and then, until the LC phase was transformed completely into the SC phase.

This description is based primarily on the discontinuities seen in the bulk deposition π-A isotherms (Figures 2, 3 and 4). A monolayer visualization study (e.g. electron microscopy or Brewster Angle Microscopy) of films formed by bulk deposition could potentially shed more light on this area.
ACKNOWLEDGEMENTS

This work was supported by the Medical Research Council of Canada (grant MT - 5462), and postgraduate fellowships from Fonds pour la Formation de Chercheurs et l'Aide à la Recherche (A.J.) and Natural Science and Engineering Research Council of Canada (R.M.P.)

REFERENCES


FIGURE CAPTIONS

Figure 1:  (a) Schematic showing the application of surfactant solution for the bulk deposition method (i) before deposition, (ii) during deposition (inevitable penetration of microsyringe needle), (iii) after deposition (DPPC in bulk as well as in monolayer).  (b) Schematic showing the application of surfactant solution for the surface deposition method (i) before deposition, (ii) during deposition, (iii) after deposition.  a - Teflon needle, b - pendant drop, c - microsyringe needle, d - surfactant solution.

Figure 2:  Three independent compressions of DPPC at 23°C using a rate of 172 Å²/molecule/minute.  Bulk deposition was used to form the monolayers.  Slight shifts in area/molecule values are due to the sensitivity of the minute amount of surfactant used in these experiments.

Figure 3:  DPPC π-A isotherms at 23 °C for the bulk deposition method using two types of spreading solvent (i.e. chloroform and hexane/ethanol).

Figure 4:  DPPC π-A isotherms for the bulk deposition method using the three samples of DPPC (Samples 1 and 2 were Approx. 99 % purity, Sample 3 was 99 + % purity.. Data for Samples 2 and 3 were obtained at 23°C and Sample 1 data was obtained at 25 °C.

Figure 5:  Three independent compressions of DPPC at 23°C using a rate of 53.3
Å²/molecule/minute. Surface deposition was used to form the monolayers. Slight shifts in area/molecule values are due to the sensitivity of the minute amount of surfactant used in these experiments.

Figure 6: DPPC π-A isotherms at 23 °C. Comparison of the bulk deposition method and the surface deposition method of monolayer formation. Each isotherm is an average of three compressions.
Fig. 1
Ph.D. Related

Publication #6
A Study of Captive Bubbles with Axisymmetric Drop Shape Analysis

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ABSTRACT

Of the many methodologies used in surface thermodynamic experimentation, Axisymmetric Drop Shape Analysis (ADSA) has proven to be one of the most accurate and versatile. Until recently, however, ADSA has had two main limitations: (i) the inability to analyze drops with near zero curvature at the apex, and (ii) problems with drop edge detection when the contrast between the drop and the background is not adequately sharp. In this paper, "second-generation" ADSA algorithms and new image analysis schemes are introduced as solutions to these shortcomings. To illustrate these improvements, an inverted sessile air bubble in a pulmonary surfactant suspension is used. This "captive bubble" geometry was chosen to overcome the problems of film leakage at low surface tensions. The modified experimental apparatus, called ADSA-Capture Bubble (CB), in conjunction with the new software, proves to be a powerful technique to investigate these types of systems. ADSA-CB is fully automated, requiring less user intervention, enabling the collection of large amounts of data, and thereby providing intricate details of surface behaviour. The so-called "squeeze-out" and film collapse phenomena are examined closely. To demonstrate the problems of film leakage, pendant (hanging) drop experimental results are also presented.
INTRODUCTION

There is an obvious need to investigate the interfacial properties of various systems. Predictions of wettability and coating, adhesion and lubrication [1], activity of protein solutions for thrombus formation [2-4], and pulmonary surfactant [5] are some of many examples. Various methodologies are used to study these systems [1, 6]: tensiometers, spinning drop, goniometers, capillary rise, Langmuir balances (LB) [7], the pulsating bubble surfactometer (PBS) [8], the captive bubble surfactometer (CBS) [9], and Axisymmetric Drop Shape Analysis (ADSA) [10-12]. Of these, ADSA has demonstrated to be one of the most versatile and accurate methods of surface thermodynamic experimentation. In the past, ADSA has been used to investigate contact angles and surface tensions of pure liquids [10], soluble mixtures [13-16], and insoluble films [17-19].

Briefly, ADSA determines the profile of axisymmetric drops and minimizes an objective function which describes the difference in the physically observed contour and the theoretical drop shape (as described by the Laplace equation of capillarity). ADSA output includes drop surface tension, area, volume, apex radius of curvature, and contact angle [10].

Although this "first-generation" ADSA has been successful in many applications [10, 13-22], it suffers from two major shortcomings. First, in some cases, the software is not able to analyze "flat" drops, i.e. situations where the curvature at the apex is near zero. In these situations, the drop's radius of curvature at that point tends to infinity, and the ADSA algorithm collapses. The occurrence of such flat drops is inevitable in situations of low and ultra-low interfacial tension. Second, it is well known that the image analysis routines used to extract the drop profile coordinates from the digital image assume sharp light contrast and fail to analyze...
At low surface tensions, pendant (hanging) drops are difficult to manipulate, because gravity overpowers surface tension and the drop falls from the support. This problem can be avoided by using a captive bubble geometry, first demonstrated by Schürch et al. [9]. In this paper we use a captive bubble of air in a surface film forming pulmonary surfactant suspension to illustrate the second-generation ADSA and new image analysis scheme.

In the presence of a surface film, the surface tension will change as the interfacial area is compressed and expanded. Without proper design, at low surface tension this type of system could potentially suffer from film leakage. Film leakage is due to a fundamental surface thermodynamic principle: at low surface tension the surface active molecules can spread from the liquid-air interface onto the surrounding solid, thereby decreasing the free energy of the system. Film leakage has been demonstrated to occur in the Langmuir-Wilhelmy film balance [23], and has been observed in the PBS [24]. The phenomenon of film leakage is a very serious problem, but has largely been ignored by many investigators. Film leakage can lead to surface behaviour which has been erroneously attributed to be intrinsic to the system.

The only way to eliminate film leakage is by removing the potential pathway through which the surface active molecules can leave the air-liquid interface. The captive bubble geometry accomplishes this by holding a bubble of air captive at the top of a chamber filled with liquid. In this system, there is no need to pierce the bubble with any capillaries. A hydrophilic ceiling ensures an aqueous layer between the solid and the bubble, leaving the air-liquid interface completely intact.

Schürch and his group [9,25-27] have demonstrated the improvements of the captive
bubble geometry over other methodologies primarily by using pulmonary surfactant solutions. They have shown that low surface tensions found with the CBS are achieved on the first compression of the interface, which is not possible with the LB or PBS [27]. Also, extremely high rates of cycling necessary in the PBS are not required for the CBS [9]. Finally, area compression ratios for the CBS are less than those required for the PBS and LB [27]. These differences are attributed to the lack of film leakage in the CBS [9,27]. The CBS uses the method of Malcolm and Elliot [28] to calculate surface tension, and uses empirical formulations to determine the bubble volume and area [29].

Although the CBS improvements over the LB and PBS are significant, further development is possible and necessary. First, the automation of bubble cycling, image acquisition, and data processing would allow for greater amounts of information to be generated. Second, in the scheme of Malcolm and Elliot, the contact angle between air and bubble is required and assumed to be 180°, not allowing for a robust methodology. Finally, multiple measurements cannot be performed readily on CBS images, and hence no indication concerning the quality of the data is available.

In this paper, we describe a new methodology, called ADSA-CB (for Captive Bubble). This technique employs the principle of the captive bubble, yet makes substantial improvements over the CBS. The improvements of the second-generation ADSA and new image analysis schemes are shown using ADSA-CB and a pulmonary surfactant solution. With this system, the problems of flat drops, fuzzy images and film leakage can be addressed simultaneously. To illustrate the problem of film leakage some pendant drop experiments are also presented.
As described above, Axisymmetric Drop Shape Analysis (ADSA) is a very accurate and flexible method to determine interfacial tensions from the shapes of sessile or pendant drops and captive bubbles [10]. A series of digital pictures of the bubble is acquired using a video camera and microscope attached to a computer with a video digitizer or frame grabber. Figure 1 shows an ADSA image of a captive bubble, i.e. an inverted sessile drop of air in a suspension of pulmonary surfactant solution.

Once the digital images are stored in computer files, image analysis software scans the images and extracts the profile coordinates of the bubble. Typically, several hundred coordinate points per image are extracted by the program, which are then given as input to numerical analysis routines that compute the interfacial tension by fitting the Laplace equation of capillarity to the profile coordinates. The entire procedure is automated, with little user intervention.

The first-generation of ADSA was implemented using the image analysis routines written by Cheng et al. [12], which employ the Sobel gradient method and cubic splines to extract the profile coordinates with subpixel resolution. However, these routines assume a sharp light contrast and fail to analyze fuzzy images such as that shown in Figure 1.

As a solution to this problem, a different image analysis scheme using image thresholding with polynomial smoothing was implemented. Despite the known limitations of the thresholding method, good results were obtained (as shown below). The Sobel edge detection and sub-pixel resolution strategies yield accuracies in the surface tension to ± 0.1 % and better. Such accuracies are not attainable by thresholding. But an accuracy of even one order of magnitude lower is quite acceptable for many applications, including the present one. Since gradient
methods do not work with bubbles in murky or semi-opaque liquids. there is no obvious alternative to thresholding. The image analysis procedure is as follows:

Once the digital images of the bubble have been saved, a rectangular area containing the bubble is defined by the user on the computer monitor. This area will be used as the working region for the image analysis. thus rejecting external objects and reducing noise that might be present in the image. Since the bubble is expanded and compressed during the course of the experiment. care is taken to ensure that the selected working area is large enough to contain the largest profiles.

A light intensity histogram of the bubble is then generated (Figure 2), which shows the distribution of grey levels of the image. This histogram is clearly bimodal, with the left peak representing the darker bubble and the right peak representing the lighter background. A grey-level threshold value smaller than the threshold value will likely belong to the bubble. A sample threshold value is shown in Figure 2.

The image is scanned in both horizontal and vertical directions (starting from the left. right, and bottom) until at least three consecutive pixels with the grey level less than the threshold value are found. The coordinates of the first of these points are then stored as the profile coordinates. The procedure continues until the whole image is scanned. Figure 3 shows the resulting profile coordinates for the drop in Figure 1. It can be seen that in addition to the profile of the bubble, some noise corresponding to dark spots in the image also appear.

To eliminate noisy points, a fifth degree polynomial is used to fit all the coordinate points found, and any point that is more than three pixels away from the fitted polynomial is rejected. This effectively eliminates the noisy points in Figure 3. By trial and error, it was found that a
fifth degree polynomial allows very good fitting to the profile of the captive bubble.

Once the profile coordinates are extracted from the image, scaling factors obtained from the image of a digitizing grid of known size are used to translate the pixel coordinates into actual dimensions.

To compute the interfacial tension, the second-generation ADSA is used [30], which uses a combination of Newton and Levenberg-Marquardt non-linear least-squares optimization techniques to fit the Laplace equation of capillarity to the profile coordinates of the bubbles. The faster but less reliable Newton method is tried first, and if it fails to converge, the Levenberg-Marquardt method is used. To take advantage of the faster convergence of the Newton method over the Levenberg-Marquardt, the optimization is initialized with an interfacial tension estimate obtained using a modified Malcolm-Elliot technique [31]. The results from the previous image are then used to initialize the next run. As opposed to the first-generation of ADSA, this software is capable of analyzing flat drops with near zero apex curvature. No limitations have been found in the second-generation, and it is considerably more efficient than the first-generation.

The second-generation ADSA is run 10 times for each image, each time using a different set of 50 arbitrarily selected profile coordinates along the entire profile, and the 95% confidence levels are compared for interfacial tension, curvature, surface area and volume, providing some indication of the image quality. The first-generation of ADSA employed 10 runs of only 20 arbitrarily selected profile coordinates, in order to save computer time.
3 APPARATUS

A schematic of the ADSA set-up is shown in Figure 4. Briefly, a CCD camera (Cohu) and microscope (Apozoom, Leitz) are used to acquire drop images. These images are stored and processed by a SPARCStation 10 workstation (Sun Microsystems). The experiments for this study involved either pendant drops or inverted sessile drops (air bubbles). The drop was illuminated by a light source (Newport) and diffuser. The temperature of the chamber housing the drop was controlled using a water bath (Lauda). The entire set-up was mounted on a vibration-free table (TMC).

In the pendant (hanging) drop experiments, a drop is formed on the end of a Teflon capillary. The other end of this capillary is connected to a syringe-plunger assembly (Hamilton). This assembly is attached to a stepper motor (Oriel) by a coupling sleeve. The motor is actuated by a controller (Oriel) so that the drop volume, and hence, the surface area is changed. The drop is enclosed in a quartz cuvette which, in turn, is kept in a temperature/pressure cell. First-generation ADSA software is used to analyze the pendant drops. For complete details on ADSA pendant drop experiments, see References [10,17].

The ADSA captive bubble chamber is comprised of two quartz viewing windows which are secured on both sides of a metal plate. A section of this middle plate has been removed, forming the side walls of the chamber (the end walls are the viewing windows). The windows are sandwiched against the metal plate by two metal end plates. Seals are ensured by O-rings on both sides of the windows. Four sets of nuts and bolts are used to fasten the whole assembly together. Two lateral holes are drilled through the end plates to allow water circulation for temperature control of the chamber. As shown in Figure 5, the section hole of the middle metal
plate has straight edges on the sides and bottom. The top was designed such that a glass piece with a concave surface could be held in place, thereby providing a glass "ceiling" for the chamber. Glass was chosen to ensure an aqueous layer between the captive bubble and the ceiling, leaving the air-liquid interface completely intact. The glass piece was obtained by cutting an optical lens.

Three ports were made to provide access to the chamber (shown in Figure 5). One port was designed for the temperature probe which remained in place during the experiment, sealing this opening. Fittings are used to connect a Teflon capillary to the second port of the chamber. The capillary, in turn, is attached to the motor-controlled syringe. The chamber internal pressure is changed by pumping liquid in/out with this assembly. The last port is used to form an air bubble in the sample chamber using a microsyringe (Hamilton) (see Methods). The software used to analyze the captive bubbles is the second-generation ADSA [30] and the new image analysis scheme described above (see Software).

4 MATERIALS

The surfactant used for this study was bovine lipid extract surfactant (BLES®. Biochemicals Inc., London, ON, Canada). Dr. David Bjarneson of BLES Biochemicals generously donated the samples. BLES is obtained by organic extraction of bovine lung lavage material. The surfactant material is extracted with chloroform:methanol and precipitated with acetone. BLES was supplied as a suspension, containing the phospholipids of natural surfactant (27 mg/ml) and surfactant associated proteins: SP-B and SP-C.

Upon supply, the vials were divided into several small aliquots of approximately 2 ml
each, capped under nitrogen and stored at -20 °C. On each day of experiments an aliquot was
removed from the freezer and gradually thawed for one hour. The suspension was gently stirred
and 1.5 ml was diluted in a 10 ml flask with 0.9% NaCl solution, resulting in a phospholipid
concentration of 400 μg/ml in the diluted surfactant solution.

5 EXPERIMENTAL PROCEDURES

5.1 Pendant Drop Experiments

A hanging drop of the diluted surfactant is formed at the end of a Teflon capillary. The
other end of the capillary is attached to the motor controlled syringe. By varying the number of
steps and the speed of the motor, the rate at which volume is removed/added to the pendant drop
can be adjusted. Hence, control over the drop volume and rate of volume change is
straightforward. ADSA pendant drop experiments are explained in detail elsewhere [10,17].

5.1.1 Adsorption

A pendant drop is formed and held at constant volume. The surface tension change with
time is observed. Images are acquired every 0.5 seconds for the first 150 seconds, then every
5 seconds for 500 seconds, and finally every 20 seconds for 50 minutes.

5.1.2 Cycling

A pendant drop is cycled at a rate of 25 seconds per cycle. This is done by programming
the motor controller to change the drop volume by an appropriate number of steps (depending
on initial drop size, corresponding to approximately 80% decrease in volume). Cycling is
continued for at least 25 cycles, or until the drop falls off. Images are acquired every 0.5
seconds during this dynamic cycling process.

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For additional experiments, the old drop is pushed off, and two other drops are formed and discarded immediately to ensure a fresh surface. A new experiment is begun with every fourth drop. All pendant drop experiments for this study were performed at 37.0 ± 0.1 °C.

5.2 Captive Bubble Experiments

The chamber is filled with the diluted surfactant solution. Using a microsyringe, an air bubble of ~15 ul is inserted into the bottom part of the chamber. The bubble then floats to the top, and comes to rest in the middle of the concave surface of the chamber ceiling.

5.2.1 Adsorption

As the air bubble is formed on the microsyringe, image acquisition begins. Time zero is taken when the bubble comes to rest at the chamber ceiling (and assumes a Laplacian shape). Images are acquired every 0.5 seconds for at least two minutes.

5.2.2 Cycling

The microsyringe port is secured and the sealing slightly compresses the air bubble. To compensate, the pressure inside the chamber is accordingly reduced by removing some surfactant suspension with the motorized syringe assembly. Five minutes are allowed to elapse to ensure complete equilibration of the system. Then, by forcing surfactant suspension into the chamber, the pressure increases, thereby compressing the air bubble/surfactant suspension interface: by removing surfactant from the chamber, the interfacial area expands. In this way, the interface can be cycled continuously. Cycling is performed at a rate of 25 seconds per cycle. The amount of compression is approximately 80% by volume, corresponding to about 65% change in surface area. It should be noted that, as with the pendant drop experiments, the number of steps used
varies with the initial drop size. Also, the amount of compression may have varied slightly between experiments. Hence, the actual rate of area change (in cm²/s) is not identical for every experiment.

To perform additional experiments, the microsyringe port is opened, and more surfactant is injected into the chamber, forcing the air bubble out of the chamber. The entire process is then repeated. All captive bubble experiments were performed at 37.0 ± 0.1 °C.

6 RESULTS

6.1 Pendant Drop Experiments

The adsorption isotherm for a typical pendant drop experiment is shown in Figure 6. The surface tension (γ) decreased quickly at first, but did not equilibrate completely, even after about one hour, when it measured 24 mJ/m².

When the pendant drop was cycled between 0.018 and 0.005 cm², a sawtooth pattern resulted for the drop volume and surface area (Figure 7). Although the surface tension response followed this general pattern, the maximum surface tension (γmax) and minimum surface tension (γmin) for each cycle decreased slightly with each progressive compression/expansion. This decrease is clearly evident by the twentieth cycle (i.e. at 500 seconds) where γmax was ~ 60 mJ/m² and γmin was ~ 17 mJ/m². This pattern seems to stay repeatable until about 600 seconds when γmax suddenly dropped to ~ 50 mJ/m² and then continued to fall while γmin stayed constant at ~17 mJ/m². Then, at about 816 seconds, γmin fell to ~15 mJ/m² (for details see Figure 8), and immediately after, the minimum volume and area (which stayed constant since the beginning of the experiment) decreased. We believe this was due to the film leakage onto the Teflon
capillary, explained in further detail in the Discussion. Figure 9 shows a composition of images before and after the film leakage. Prior to the leakage, the three phase line, where the capillary and drop intersect, was easily discernable (Figure 9 (a), (b), and (c)). Immediately after leakage (Figure 9 (d)) this intersecting line was obscured.

Figure 10 shows the results of another pendant drop compression/expansion experiment (volume cycled between 0.018 and 0.004 cm³). As before, each progressive cycle results in a lower γₘᵢₙ and γₘₐₓ. Upon the first compression a slight "shoulder" is seen to occur at approximately 22 mJ/m². This shoulder eventually disappears with progressive cycling. On the second and subsequent cycles of Figure 10 an additional shoulder is seen at about 15 mJ/m². In later cycles, γ is even seen to increase in this region before it starts decreasing again. We believe this second plateau is another, more subtle, manifestation of film leakage, explained in detail in the Discussion.

6.2 Captive Bubble Experiments

Figure 11 shows the adsorption process for the captive bubble system. Compared to the relatively slow equilibration of the pendant drop experiments, the equilibrium surface tension (γₑ) in captive bubble experiments was reached in about 25 seconds with a value of 22.7 mJ/m². Dynamic cycling was begun after the system was equilibrated (i.e. 5 minutes after sealing the microsyringe port). Figure 12 is a composition of drop volume, surface area and γ as a function of time. The periodic pattern is obtained by controlling the chamber pressure, resulting in a linear increase and decrease in volume and area, forming a sawtooth response. It should be noted that the initial and final values of the area and volumes for the air bubble in these experiments are similar to the values of area and volume the pendant drop experiments (shown in Figure 7).
The number of cycles per minute are identical for both types of experiments.

Several interesting features are exhibited by the surface tension response to dynamic cycling. For clarity, Figure 13 shows the first three cycles of a typical run. First, it should be noted that the cycles begin at the \( \gamma_{eq} \) value of \(-23 \text{ mJ/m}^2\) as described above. Upon compression of this equilibrated film, a slight shoulder is exhibited at \(-21 \text{ mJ/m}^2\). Further compression on this first cycle yields surface tensions in the order of \(2 \text{ mJ/m}^2\) (for details see Figure 14). This compression was stopped prior to film collapse since lower \( \gamma_{min} \) values are possible (see below).

Upon the first expansion (Figure 13), the surface tension rises well above that of \( \gamma_{eq} \), to \(-29 \text{ mJ/m}^2\). The surface tension increases rapidly when the interface expands, \( \gamma \) increasing most rapidly, to approximately \(27 \text{ mJ/m}^2\), within the first few seconds of expansion.

A shoulder (at \(-21 \text{ mJ/m}^2\)) can still be detected in the second compression of Figures 12 and 13, but dampens out as the cycling progresses, and by the twentieth cycle (Figure 12) it is no longer observable. Some of these shoulders, however, are quite subtle, as shown in the second cycle of Figure 13 and in detail in Figure 15. When the data points are fit with linear regression lines, a shift is clearly seen.

It should be noted that the error bars shown in the figures are the 95% confidence limits of the surface tension. As explained above (see Software), these error limits are determined by running the ADSA software 10 times for every drop image, each time using a different set of 50 arbitrarily selected profile coordinates.

Figure 16 shows three dynamic cycles of an experiment where the interface was compressed sufficiently to achieve collapse of the surface film. Although not entirely obvious in this diagram, close examination of a region of minimum surface tension (Figure 17) showed
an intricate pattern. At 523.5 sec, \( \gamma \) was approximately 0.5 mJ/m\(^2\), and half a second later increased to \(-1.5\) mJ/m\(^2\). At 524 sec, the interface was still being compressed and between this time and one second later the surface tension decreased to, and remained at, \(-1.0\) mJ/m\(^2\). Then between 525 and 526.5 seconds, when the bubble was between compression and expansion, ADSA failed to provide data, despite the images being acquired. This might be due to the bubbles being non-Laplacian in shape (see Discussion).

Figure 18 shows a composition of the images acquired during the compression/expansion cycle shown in Figure 17. The numbered points in Figure 17 correspond to the appropriate bubble images shown in the composition.

7 DISCUSSION

Despite the use of the same surfactant suspension (diluted bLES\(^\text{®}\), 400 \(\mu\)g/ml), obvious differences exist between the pendant drop and captive bubble experimental results. The adsorption equilibration time for the pendant drop was many times that for the captive bubble. Although the interfacial area in both cases were approximately equal (\(-0.30\) cm\(^2\)), the volume of liquid in the captive bubble chamber was about a hundred times larger than the volume of the pendant drop. Hence, we suspect that the greater amount of suspension provides greater availability of surfactant, allowing the captive bubble system to equilibrate faster. Also, there was obvious surfactant suspension convection seen in the captive bubble chamber. It is not clear whether this type of mixing was occurring inside the pendant drop. Reduced mixing could provide another explanation as to why the pendant drop required so much more time to equilibrate.
In the dynamic cycling experiments for pendant drops, the $\gamma_{\text{max}}$ and $\gamma_{\text{adv}}$ drifted down with progressive cycling, until film leakage occurred. This leakage occurred at about 15 mJ/m$^2$. Despite the similar compression ratios and rates used in both types of experiments, a $\gamma_{\text{max}}$ of -1 mJ/m$^2$ was measured in the captive bubble system on the first cycle (Figure 16); $\gamma_{\text{max}}$ observed for captive bubble apparatus was only about 29 mJ/m$^2$ as opposed to 38 mJ/m$^2$ (after film leakage) in the pendant drop experiments.

These considerations, as well as the clear evidence of material being lost, indicated by a volume decrease (Figure 7) and the actual images of leakage (Figure 9), clearly illustrate the shortcomings of the pendant drop experiments for this type of system. The film leakage problem has been suspected for many years and has been discussed in detail before [23,24,26,27]. For example, Goerke and Gonzales used radio-tracers to show that a DPPC film leaked and coated the walls of a Langmuir-Wilhelmy trough [23]. Putz et al. [24] reported that they were able to observe film leakage by viewing the capillary supporting the air bubble in the pulsating bubble surfactometer, but no images were included in their paper. In the pendant drop experiments shown in Figure 7, when film leakage occurred, ADSA detected a loss of drop volume because some liquid moved above the critical cut-off point at the end of the capillary which provides the upper limit for ADSA image analysis.

The onset of leakage reported in this study was found to occur at $\sim$15 mJ/m$^2$. This makes intuitive sense from the point of view of surface energetics. The surface tension of Teflon in air is approximately 18 mJ/m$^2$ [32]. Presumably then, when the interfacial tension falls below this value, the leakage is thermodynamically favoured, since it will reduce the free energy of the system.
It is clear that the captive bubble apparatus, which eliminates potential pathways for leakage, circumvents such problems. Although the captive bubble surfactometer (CBS) has been shown to be effective [9,24], this newer ADSA methodology (ADSA-CB) has several advantages. First, ADSA-CB is completely automated including image acquisition and processing. Hence, user skill is not critical, making this methodology less subjective. As opposed to the CBS, which is time consuming [24], measurements with ADSA-CB are straightforward. Second, the errors with ADSA-CB are typically less than those with other conventional methods [10]. Third, with the CBS there is no indication of the data quality. The multiple measurements of surface tension on one image using ADSA is done in order to calculate the 95% confidence levels. These provide indications of image quality and/or the deviation from Laplacian shape (i.e. when the system is not at mechanical equilibrium). Fourth, because of the ease of data acquisition and processing with ADSA, many data points are obtainable. A typical experiment is comprised of hundreds of data points, resulting in many thousands of processed images for this study alone. Finally, because of the vast amount of data generated with ADSA, details are discernable now which were not amenable to observation before. The large amount of measurements obtainable with ADSA allows the experimenter to observe minute intricacies of the surface behaviour.

The second-generation of ADSA and the new image analysis schemes overcome the two major shortcomings plaguing the older version of ADSA. The edge detection routines employed here effectively handle the fuzzy images of an air bubble in a murky suspension, and the ADSA software is able to handle the flat bubbles obtained at low surface tensions.

An example of the details exhibited by ADSA-CB in conjunction with the second-generation ADSA and new image analysis schemes, is the shoulder which occurs at -21 mJ/m². 

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This shoulder has been attributed to the so-called "squeeze-out" phenomenon [5,25]: compression of the interface causes non-DPPC components to be removed from the surface. In our experiments, this phenomenon was less noticeable after the first cycle but still occurred, as shown in detail in Figure 15. As Schürch et al. have shown [25], this feature eventually disappears as the surface film is enriched in DPPC. Figure 15, however, shows that although not completely obvious, squeeze-out still occurs after many cycles.

Another region of the isotherm that demonstrates the advantage of detailed data is that where collapse occurs. In Figure 17, when \( \gamma \) reached \(-0.5\ \text{mJ/m}^2\), it is likely that expulsion of DPPC molecules from the film (i.e. collapse) occurred, resulting in a surface tension increase of about \( 1\ \text{mJ/m}^2 \). Further compression resulted in a lowering of \( \gamma \) once again. On a larger scale (and with less detail), these fluctuations would perhaps be missed, resulting in rather flat plateaux at low \( \gamma \), the widely accepted indication of film collapse.

It should also be noted that violent abrupt bubble movements were observed and recorded on video as the interfacial film collapsed. The rather large error limits at \( \gamma = 0.5\ \text{mJ/m}^2 \) in Figure 17 may indicate that the bubble was in the midst of one of these sudden movements, and close to being non-Laplacian in shape. In addition, in the interlude between the compression and expansion (Figure 17), the images could not be processed by ADSA, again indicating the deviation of the bubble shape from mechanical equilibrium. If this is case, the Malcolm-Elliot method of determining drop surface tension would not detect anything amiss.

The captive bubble isotherms at the maximum surface tension show a very characteristic shape. Upon expansion of the interface, the surfactant concentration is diluted, and hence, the surface tension increases very rapidly. This sudden increase in surface tension (above \( \gamma_m \))
provides a strong impetus for adsorption, resulting in a flat surface tension region in the isotherm. These results indicate the rapid adsorption of surfactant when the interface is expanded and the surface tension is above the equilibrium value. The preliminary data indicates that the rate at which the interfacial area is increased effects $\gamma_{\text{max}}$. We found (data not shown) that slightly slower rates of area change result in a $\gamma_{\text{max}}$ slightly closer to $\gamma_{\text{eq}}$. This makes intuitive sense since at infinitely slow rate of area change (i.e. quasi-static), $\gamma_{\text{max}}$ should be limited to $\gamma_{\text{eq}}$.

ACKNOWLEDGEMENTS

This research was funded by the Medical Research Council of Canada (MT-5462).
FIGURE CAPTIONS

Figure 1: Image of a captive bubble as reproduced by laser printer.

Figure 2: Light intensity histogram showing the grey levels of the image in Figure 1. A sample thresholding value is also included.

Figure 3: Profile coordinates for the drop shown in Figure 1. Note the presence of noise.

Figure 4: Schematic of the ADSA step-up. (1) Drop cell/bubble chamber, (2) Teflon capillary, (3) water bath, (4) temperature probe, (5) light source, (6) diffuser, (7) microscope, (8) CCD camera, (9) Sun workstation, (10) motor controller, (11) syringe assembly, (12) stepper motor.

Figure 5: Schematic of the section hole of the middle metal plate forming the side walls of the ADSA-CB test chamber. (1) Captive bubble, (2) pulmonary surfactant solution, (3) glass ceiling, (4) microsyringe port, (5) syringe port, (6) temperature probe port.

Figure 6: Adsorption isotherm for the pendant drop experiment.

Figure 7: Dynamic cycling for the pendant drop experiment. Composite of drop volume, surface area and surface tension as functions of time.

Figure 8: Detailed surface tension as a function of time, for the time frame of 800 to 840 seconds of Figure 7. Film leakage occurred at about 816 seconds (see text for details).

Figure 9: Composition of images of pendant drops as reproduced by laser printer. Demonstration of leakage: (a), (b), and (c) are prior to leakage, the Teflon capillary is clearly discernable; (d) is after leakage, the Teflon capillary is covered by the surfactant solution.

Figure 10: Dynamic cycling for another pendant drop experiment; surface tension as a function of time.
Figure 11: Adsorption isotherm for the captive bubble experiment.

Figure 12: Dynamic cycling for the captive bubble experiment. Composition of drop volume, surface area and surface tension as a function of time.

Figure 13: Surface tension as a function of time for the first three cycles of a captive bubble experiment. The error limits shown are the 95% confidence levels.

Figure 14: Detailed surface tension as a function of time. Low surface tension region of the first cycle in Figure 13. The error limits shown are the 95% confidence levels.

Figure 15: Detailed surface tension as a function of time. The shoulder region of the second cycle in Figure 13. The error limits shown are the 95% confidence levels.

Figure 16: Surface tension as a function of time for the first three cycles of a captive bubble experiment. Notice that these compressions were sufficient to achieve collapse. The error limits shown are the 95% confidence levels.

Figure 17: Detailed surface tension as a function of time of a collapsing film. The missing points are probably due to the non-Laplacian shape of the bubble (see text for details). The error limits shown are the 95% confidence levels.

Figure 18: Composition images of the captive bubble as reproduced by laser printer. 1, 2 and 3 correspond to Points 1, 2 and 3, respectively, of Figure 17.
REFERENCES


Fig 6
Ph.D. Related

Publication #7

(in preparation)
The Correlation Between Phospholipid Content and Surface Tension of Pulmonary Surfactant from Lavaged Rabbits

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ABSTRACT

Samples of pulmonary surfactant were obtained from rabbits by repeated lung lavage. Surface tension measurements of the samples were made in such a way as to avoid the possibility of film leakage. While this method provides no information about the minimum surface tension that can be attained during compression, the relative magnitudes of the surface tension values are strongly correlated to the amount of phospholipids in the samples. Furthermore, the surface tension measurements are a more sensitive means to detect the difference between the lavage samples than the total phospholipid content.
INTRODUCTION

In has been known since the pioneering work of Avery and Mead (1959) that the main contributor to Respiratory Distress Syndrome (RDS) is a lack of pulmonary surfactant. In order to investigate possible treatments for RDS, a useful model for this disease is the in vivo, whole lung lavage model (c.f., for example Lachmann et al. (1980), Kuckelt et al., (1981), and Kobayashi et al., (1984)). The effect of the lavage in these studies can be clearly seen in the change in the PaO₂ readings before and after the lavage.

An alternate method to characterize both the degree of lung injury induced by the repeated lung lavages and the degree of success of the treatment is to measure the surface activity of the lavage fluid. However, there are two potential difficulties in using this procedure: First, the surface activity is strongly dependant on the composition of pulmonary surfactant (Norter and Finkelstein, 1984). Therefore, some indicator of the composition of the lavage fluid must also be measured. Second, the most commonly used methods for measuring pulmonary surfactant activity, the Langmuir-Wilhelmy balance (as first used by Clements (1957)) and the pulsating bubble surfactometer or PBS (Enhorning, 1977) suffer from experimental artifacts due to film leakage (Goerke and Clements, 1986). For example, once the surface tension of the pulmonary surfactant drops below that of the plastic tube of the PBS (~18 mJ/m² for Teflon), the surface film may preferentially spread on to the tube (Schürch, 1993). This has the net effect of a decrease in the actual amount of film compression which results in higher values of surface tension (Putz et al., 1994a).

In this study, we describe a method for measuring surface tension of lavage material that avoids the problem of film leakage and demonstrate that the relative magnitudes of the surface
tensions measured by this technique are correlated to the total amount of phospholipids in the sample. Although this procedure does not provide any information about the minimum surface tensions that can be attained during compression, they may be a useful yardstick to investigate the effect of lavage.

EXPERIMENTAL

Acquisition of the Samples

Lavage material was obtained from three adult New Zealand white rabbits using the protocol of Lachmann et al. (1980). After a control period of 30 minutes of normal ventilation at PEEP = 0 and \( V_T = 10 \) ml/kg, repeated lung lavages with aliquots of 25 ml/kg of warmed normal saline were performed. This procedure was repeated until the \( \text{PaO}_2 \) was reduced to below 60 mm Hg 15 minutes after the lavage (PEEP = 5 cm H\(_2\)O, \( V_T = 5 \) ml/kg, \( \text{FiO}_2 = 1.0 \), respiratory rate = 30/minute). The animals were then ventilated for four hours under the above conditions (with exception of Rabbit #1 which had \( V_T = 10 \) ml/kg) and then a final lavage was taken.

A total of nine samples of lavage material were studied. The material from the first lavage, the lavage immediately before ventilation, and the lavage immediately after ventilation were taken from each animal and were denoted as the baseline, before-ventilation, and after-ventilation washes respectively. Each sample was centrifuged at 150 x g for 10 minutes at room temperature to remove cellular debris. The supernatant liquid was then centrifuged at 40,000 x g for 15 minutes at 4 °C. The resulting pellet was suspended in 0.075 M NaCl and divided into two aliquots for phosphorous assay and surface tension measurements.
Phospholipid Content

The total phosphorous content of each sample was determined by the method of Rouser et al. (1970). The total amount of phospholipid was then estimated by multiplying the phosphorous content by 25 (Collacicco et al. (1977); Tanaka and Takei (1983); and Putz et al. (1994)). This procedure assumes that the average molecular weight of the phospholipids is 775.

Surface Tension Measurements

The surface tensions of the samples were measured by a technique called Axisymmetric Drop Shape Analysis - Profile (ADSA-P) which is shown in the schematic diagram in Figure 1. A pendant drop of pulmonary surfactant was formed on the end of a vertical Teflon capillary of circular cross-section. The volume of the pendant drop was controlled by the stepper motor (Model 18515, Oriel, Stratford, CT, USA) which was attached to the plunger of a syringe (Gastight Syringe, Hamilton Co., Reno, NV, USA) connected to the other end of the capillary. The drop was lowered into a quartz cuvette (Helma, Concord, ON, Canada) which was enclosed in an environmental chamber (Model 100-07, Rame Hart Inc., Mountain Lakes, NJ, USA) to isolate the drop from the surroundings. The temperature of the chamber was controlled to 37.0±0.1 °C by a thermostatted water bath (Model K-2/R, Lauda, Germany). The pendant drop was illuminated by a white light source (Model V-WLP 1000, Newport Corp., Fountain Valley, CA, USA) shining through a heavily frosted diffuser. Using a microscope (Leitz Apozoom, Leica, Willowdale, ON, Canada) and a charged-coupled device video camera (Cohu 4810, Infrascan, Inc., Richmond, BC, Canada), images of the pendant drop were digitized to an image of 640x480 pixels with 246 grey levels by a digital video processor (Parallax XVideo Board, ).
The present set-up is able to acquire images at a rate of up to 2.5 images per second. The digitized images were acquired from the digitization board by a Sun SPARCstation 10 workstation. The entire set-up was mounted on a vibration-free table (Technical Manufacturing Corp., Peabody, MA, USA) to minimize the effects of external vibrations.

The determination of the surface tension of the pendant drop is described in detail by Rotenberg et al. (1983), Cheng et al. (1990) and Cheng and Neumann (1992). Briefly, ADSA-P software determines the experimental drop profile coordinates to sub-pixel resolution with image analysis techniques. Theoretical drop profiles are computed from the Laplace equation of capillarity and non-linear regression techniques are used to minimize the deviation of the theoretical drop profiles from the experimental coordinate points to calculate the surface tension, area, volume, and radius of curvature of the experimental drop.

Although ADSA-P has never been used to measure the surface tension of pulmonary surfactant, it has several features which make it well-suited for this application. ADSA-P has been used recently to measure the interfacial properties of insoluble monolayers (Kwok et al., 1994) and soluble protein solutions (Miller et al., 1993). Since ADSA-P is highly automated, it is less dependant on operator skill and has the potential to generate a significant amount of data. Furthermore, the amount of sample material required is very small (the pendant drops used in this study were approximately 0.02 cm³). Finally, it is possible to vary independently the compression speed and the compression ratio during dynamic cycling of the pendant drop, which is not possible with the PBS (Enhoring, 1977).

Measurements of surface tension were completed on static and dynamic drops. In the static drop experiments, the surface tension was monitored as a function of time during the
equilibration process of a pendant drop of constant volume. Measurements were made at a rate of once every ten seconds over a 15 minute period for each of three experimental runs with each sample. After this period, the surface tension was changing at an average rate of only 0.0017 mJ/m²/s and the final surface tension reading was taken to be the equilibrium value. In the dynamic drop experiments, a fresh pendant drop was formed and volume was changed in a linear manner with time in such a way that the average rate of compression was 0.0199 ± 0.0009 cm²/s. The drop was compressed only once and the surface tension was not allowed to equilibrate before the compression. Therefore, these dynamic measurements reflect the combined effects of surfactant adsorption and film compression.

**Statistical Analysis**

All data reported are means ± 95% confidence limits. Two factor analysis of variance (ANOVA) tests were performed to investigate the effect of the washing and the source rabbits on phospholipid content and surface tension. Differences were considered to be statistically significant at a level of p < 0.05.

The correlation between two variables was achieved by the rank correlation test. This method is particularly robust since it does not make any assumptions about the functional relationship between the two variables, nor does it make any assumptions about the underlying probability density functions of the variables (Walpole and Myers, 1978). Two variables were considered to be significantly correlated at a level of significance of p < 0.05.

**RESULTS**
Phospholipid Content

The total phospholipid content of the nine samples are summarized in Table 1. It was found that the source rabbit did not have a statistically significant effect \((p = 0.274)\), while the wash did have a statistically significant effect \((p = 0.027)\). In general, the highest phospholipid concentration was found in the baseline washes, followed by the after-ventilation washes, and then the before-ventilation washes.

Equilibrium Surface Tension

The results of the equilibration experiments are shown in Figure 2 and the resulting equilibrium surface tensions are shown in Table 2. Note the high degree of repeatability of the surface tension measurements. Both the wash and the source rabbit had a statistically significant effect on the equilibrium surface tension \((p < 0.001)\).

Dynamic Surface Tension

Typical plots of the variation in volume, area, and surface tension of a sample during the dynamic drop experiments are shown in Figure 3. Similar data for each sample was then converted into surface tension-area isotherms. This data was then interpolated to equal relative areas using cubic spline interpolation so that it was possible to average the three experimental runs completed for each sample. The compression portions of the resulting surface tension-area isotherms are shown in Figure 4.

We arbitrarily selected the values of surface tension at a relative area of 0.75 to perform statistical analysis. (Since the surface tension-area isotherms shown in Figure 4 do not intersect,
the relative area at which the values are selected will change the absolute values, but will have no effect on the relative magnitudes.) Both the wash and the rabbit had a statistically significant effect on the dynamic surface tension (p < 0.001).

**Correlation Between Phospholipid Content and Equilibrium Surface Tension**

Figure 5 shows a plot of equilibrium surface tension as a function of the amount of phospholipid in the samples. As can be seen in the figure, there is a nonlinear trend of decreasing surface tension with increasing phospholipid content.

This trend was quantified by the rank correlation test. It was found that there was a high correlation between the equilibrium surface tension and the phosphorous content of the samples: the levels of significance for the three runs were 0.022, 0.020, and 0.020, while the level of significance for the average of the three runs was 0.030. In other words, we are certain with at least 97% confidence that the equilibrium surface tension is correlated to the amount of phospholipid in the sample.

**Correlation Between Phospholipid Content and Dynamic Surface Tension**

The surface tensions at the relative area of 0.75 are plotted in Figure 6 versus the phospholipid content of the samples. Once again the general trend of a decrease in surface tension with an increase in phospholipid content is evident. The levels of significance of the rank correlation for the three experimental runs were < 0.001, 0.017, 0.020, while the level of significance for the average of the three runs was 0.012. Therefore, we are certain with at least 98% confidence that the phospholipid content is correlated to the dynamic surface tension.
Correlation Between Equilibrium and Dynamic Surface Tension

The correlation between the two types of surface tension measurements was calculated to determine whether or not they were consistent. The level of significance of the rank correlation between the average of the three runs for each type of measurement was < 0.001 so that we are certain with > 99.9% confidence that the two type of surface tension measurements are correlated.

DISCUSSION

As can be seen in Figure 2, the equilibrium surface tensions of the baseline washes were near 25 mJ/m², which is the equilibrium surface tension of pulmonary surfactant in the presence of an excess of phospholipid (Goerke and Clements, 1986). However, the equilibrium surface tensions of the before-ventilation and after-ventilation samples were generally larger. It is not likely that this could be due to insufficient phospholipid since Schürch et al. (1992) and Schürch et al. (1994) showed that the equilibrium surface tension of pulmonary surfactant was ~25 mJ/m² even at a total phospholipid content as low as 50 µg/ml.

An alternate explanation is that processes of repeated lung lavages and prolonged ventilation induced the release of blood proteins in to the lung. It has been demonstrated that blood proteins such as fibrinogen, albumin, and bilirubin inhibit the surface activity of pulmonary surfactant and phospholipid monolayers (c.f., for example, Tierney and Johnson (1965); Tabak and Notter (1977); Collacicco and Basu (1978); Notter et al. (1982); Seeger et al. (1985); and Fuchimukai et al., 1987). Therefore, the repeated lavages and/or the prolonged ventilation may
have caused bleeding in the lungs and blood proteins would have become part of the sample composition. To the authors' knowledge, there has not been any previous research into the change in composition of lavage fluid during repeated lavage. However, the fact that some of the samples had a reddish tinge provides indirect evidence that there was a lung injury that resulted in bleeding of the respiratory tract.

Thus, the process of repeated lung lavage decreases the phospholipid content and it is reasonable to assume that it increases the blood protein content; both of these changes in lavage composition would increase the magnitude of the surface tension. Nonetheless, the experimental data shows that the total phospholipid is highly correlated to the relative magnitudes of the surface tension measurements, so that a relatively simple measurement of the total phospholipid is sufficient to characterize the sample composition with regards to surface activity. Further investigation on the change in the protein composition during repeated lavage would be required to gain a better understanding of the change in surface activity during repeated lavage.

Because the liquid-air interface of a pendant drop is pierced by the Teflon capillary, there exists a possible pathway for film leakage to occur. However, the two experimental procedures outlined in this study were specifically designed to avoid film leakage and the experimental results indicate that no significant film leakage occurred: First, the equilibrium surface tensions were greater than that of the Teflon capillary so that there would be no driving force for the film to spread on to the capillary. At the relative area of 0.75, the surface tensions were also greater than that of the Teflon capillary. Second, the statistical analysis indicates that the equilibrium surface tension and the dynamic surface tensions are correlated with > 99.9% confidence. Since it is extremely unlikely that film leakage would have occurred during equilibration, we can
reasonably assume that film leakage effects during dynamic compression were also negligible. Therefore, although neither of the described procedures provides information about the minimum surface tension that can be obtained for a given sample, they are a useful "benchmark" test to compare the surface activity of different samples.

The usefulness of the surface tension measurement procedures can be demonstrated by the sample data. According to the phospholipid content, there was no statistically significant difference between the source rabbits and it would have been valid to pool the samples from the different rabbits for each wash. On the other hand, according to both sets of surface tension measurements, there is a statistically significant difference between the source rabbits. As can be seen in Figures 2 and 4, the 95% confidence intervals for the surface tensions do not generally overlap and therefore the readings are statistically different. Furthermore, Figures 5 and 6 show that the resulting surface tensions of the samples are highly nonlinear at the low phospholipid concentrations: for a small change in the amount of phospholipid, there is a very large change in the measured surface tension. Therefore, the surface tension measurements are a much more sensitive means to detect the difference between the samples than the phospholipid measurements.

It is not possible to measure the surface tensions during the equilibration process with the PBS. Although it is impractical to measure adsorption kinetics with the slowly responding Langmuir-Wilhelmy balance (King and Clements, 1972), an equilibrium surface tension could be determined after waiting a sufficiently long time. However, the dynamic surface tension method as described in this study could be employed with both of these surface tension measurement techniques. A fresh bubble or film would be formed and immediately compressed and the surface tension at a fixed amount of compression could be used as a yardstick to compare
different samples from repeated lavage. This information could then be used to quantify the
degree of lung injury induced by the lavage procedure and the degree of recovery after treatment
by comparing the surface activity of different washes.

SUMMARY AND CONCLUSIONS

The relative magnitude of the surface tension of a sample of pulmonary surfactant is
strongly correlated to the magnitude of the total phospholipid content under both static and
dynamic conditions. This indicates that the concentration of phospholipids is sufficient to
characterize the composition of samples with regards to surface activity. Although this procedure
does not provide information about the minimum surface tension, the surface tension values are
a more sensitive means to detect the difference among many samples than the phospholipid
composition.

ACKNOWLEDGEMENTS

This work was completed under MRC grant MT-5462. In addition, we wish to
acknowledge NSERC for a postgraduate scholarship to support R.M.P. and Fonds FCAR for
financial support of A.J.
REFERENCES


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Tanaka, Y., and Takei, T. "Lung surfactants : I Comparison of surfactants prepared from lungs


### Table 1: The phospholipid content of the nine samples in mg/ml.

<table>
<thead>
<tr>
<th>Wash</th>
<th>Rabbit 1</th>
<th>Rabbit 2</th>
<th>Rabbit 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>4.38</td>
<td>6.29</td>
<td>12.2</td>
</tr>
<tr>
<td>Before-ventilation</td>
<td>0.398</td>
<td>0.549</td>
<td>1.32</td>
</tr>
<tr>
<td>After-ventilation</td>
<td>0.920</td>
<td>0.891</td>
<td>1.55</td>
</tr>
</tbody>
</table>

### Table 2: The equilibrium surface tensions of the nine samples in mJ/m². Values are means ± 95% confidence intervals for three experimental runs.

<table>
<thead>
<tr>
<th>Wash</th>
<th>Rabbit 1</th>
<th>Rabbit 2</th>
<th>Rabbit 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>30.3 ± 1.98</td>
<td>34.0 ± 1.70</td>
<td>26.5 ± 0.82</td>
</tr>
<tr>
<td>Before-ventilation</td>
<td>34.9 ± 0.86</td>
<td>46.0 ± 1.06</td>
<td>45.5 ± 2.28</td>
</tr>
<tr>
<td>After-ventilation</td>
<td>41.4 ± 1.52</td>
<td>38.3 ± 1.79</td>
<td>29.9 ± 1.78</td>
</tr>
</tbody>
</table>

### Table 3: The surface tension of the nine samples after a compression by 25%. Values are means ± 95% confidence intervals for three experimental runs.

<table>
<thead>
<tr>
<th>Wash</th>
<th>Rabbit 1</th>
<th>Rabbit 2</th>
<th>Rabbit 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>10.6 ± 3.37</td>
<td>26.8 ± 1.21</td>
<td>14.2 ± 0.727</td>
</tr>
<tr>
<td>Before-ventilation</td>
<td>40.8 ± 1.00</td>
<td>52.0 ± 5.86</td>
<td>41.5 ± 8.29</td>
</tr>
<tr>
<td>After-ventilation</td>
<td>43.5 ± 2.94</td>
<td>40.3 ± 1.42</td>
<td>32.1 ± 1.62</td>
</tr>
</tbody>
</table>
FIGURES

Figure 1: A schematic diagram of the ADSA-P set-up: (1) pendant drop; (2) Teflon capillary; (3) quartz cuvette; (4) environmental chamber; (5) water bath; (6) white light source; (7) diffuser; (8) microscope; (9) CCD camera; (10) Sun workstation; (11) syringe; (12) stepper motor; (13) stepper motor controller.

Figure 2: Surface tension measurements during the adsorption process: (A) baseline washes; (B) before-ventilation washes; (C) after-ventilation washes. The symbols correspond to the source rabbits: (●) Rabbit 1; (■) Rabbit 2; (◆) Rabbit 3. All values are means ± 95% confidence levels for three experimental runs.

Figure 3: A typical ADSA-P result for the dynamic surface tension measurements. Three experimental runs are shown for the baseline wash of Rabbit 2.

Figure 4: The dynamic surface tensions after a compression by 25%. The letters A, B, and C and the symbols have the same meaning as Figure 2.

Figure 5: The variation in equilibrium surface tension with phospholipid content of the nine samples.

Figure 6: The variation in dynamic surface tension with phospholipid content of the nine samples.
OBaseline
Before Ventilation
A After Ventilation

Phospholipid Content (rng/m)

Surface Tension (mJ/m²)

Phospholipid Content (mg/ml)

Fig. 5
Before Ventilation

After Ventilation

O 5
10
15
Phospholipid Content (mg/ml)

Surface Tension (mJ/m²)

- Baseline
- Before Ventilation
- After Ventilation

Phospholipid Content (mg/ml)
Ph.D. Related

Publication #8

(in preparation)
Thermodynamic Modelling of the Lung

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Abstract

From a thermodynamic point of view, we obtain a general expression of the distortion energy. From a Maxwell relation for this energy expression, we obtain a relation between the recoil pressure and the surface tension. By using the properties of partial derivatives, we derive a general thermodynamic expression of the surface area. For the two separate cases: 1) lungs with constant surface tension and 2) air-filled lungs, we provide explicit expressions for the surface area, the distortion energy and mechanical work by obtaining the fitting parameters upon comparing the model to the experimental data. The model prediction is in good agreement with the experiment in all situations.
Introduction

In lung surfactant studies, several quantities are important in order to understand the lung function and its relation to microstructures of lung parenchyma: alveolar surface tension, surface area, volume and recoil pressure. In addition, the relations among these quantities are necessary to understand of the micromechanics of peripheral air space. However, it is difficult to obtain both surface tension data and pressure-volume information simultaneously from the experiment. To correlate \textit{in vitro} surface tension-area measurements with \textit{in vivo} pressure-volume observations, it is necessary to build a lung model. Such a model would, for example, predict the surface tension and infer the status of the lung lining from the pressure-volume PV diagram of a diseased or injured lung.

Earlier attempts at lung modelling [1-6] depend on rather detailed assumptions about alveolar geometry and the contributions of tissue and surface tension forces to lung recoil. Bachofen et al. [1] assumed that alveolar geometry remains similar as lung volume changes and that surface area varies with a two-thirds power of lung volume. They further assumed that the tissue contribution to recoil pressure in the air-filled lung equals the recoil pressure of the saline-filled lung at the same lung volume. The additional work of inflating the air-filled lung was set equal to the surface energy to calculate surface tension. Valberg and Brain [7] eliminated some of the necessary assumption of volume-area relation but used geometrical equality at equal volume between the fluid-filled and the air-filled lungs. More recently, Wilson [8-10] proposed a method for calculating surface tension that relies on only one assumption that the tissue elements of the lung form a conservative mechanical system with an elastic energy which can be described as a function of volume and surface area. His model does not assume that the tissue component of recoil in the air-filled lung is the same as the
recoil of the saline-filled lung. Instead, he stated that the total of the tissue and surface energies is minimum at equilibrium. A significant result of this model is that the tissue component of the recoil in the air-filled lung is greater than the recoil of the saline-filled lung. However, in predicting the difference between recoil pressure of air- and saline-filled lungs as a function of surface area, an empirical equation was used to fit experimental data. This prompted us to analyze the lung mechanics from a thermodynamic point of view, utilizing principles of thermodynamics as a guideline to obtain relevant functional relationships among different variables.

The basic principle of our model is to examine the theoretical thermodynamic requirements for a stable lung configuration. Beginning with the fundamental equation of thermodynamics and considering the mathematical condition of exactness, we derive expressions among surface tension, surface area, pressure and volume of the lung. For example, we can derive an expression for the lung surface area as a function of lung volume and surface tension. In this paper, we first present the thermodynamic foundation of the model and then provide the derivations of expressions for lung area, surface tension and the work associated with lung inflation and deflation. Finally, we apply the model to experimental situations and compare it with experimental data.

**Thermodynamic Analysis of the Lung**

Suppose that the total energy of the lung at a given configuration is $U$. It is not possible to measure $U$ directly, so we can write

$$U = U_{\text{reference}} - U_{\text{distortion}}$$  \hspace{1cm} (1)

4
where $U_{\text{assum}}$ is the change in energy of the lung from some reference condition. A convenient reference state is the condition where the surface tension of the lung $\gamma = 0$ and the volume $V = 0$. Therefore, $U_{\text{assum}}$ represents the change in lung energy due to a) the change in volume and b) the change in area, i.e.,

$$U = U(V, A)$$  \quad (2)$$

The differential form of this equation

$$dU = \left( \frac{\partial U}{\partial V} \right)_A dV - \left( \frac{\partial U}{\partial A} \right)_V dA$$  \quad (3)$$

The following parameters are defined with the condition that positive work is done on the lung when increasing the lung volume and surface area

$$P_\gamma = \left( \frac{\partial U}{\partial V} \right)_A \quad \text{and} \quad \gamma = - \left( \frac{\partial U}{\partial A} \right)_V$$  \quad (4)$$

Here $P_\gamma$ is the recoil pressure due to the non-zero surface tension, i.e., $P_\gamma = P - P_{\gamma 0}$ with $P$ as the transpulmonary pressure at non-zero surface tension and $P_{\gamma 0}$ at the zero surface tension. Thus, Eq. (3) becomes

$$dU = P_\gamma dV - \gamma dA$$  \quad (5)$$

Consider the second derivative of $U$, by an elementary theorem of calculus, the order of differentiation can be changed for well-behaved mathematical functions, i.e.,
\[ \frac{\partial^2 U}{\partial V \partial A} = \frac{\partial^2 U}{\partial A \partial V} \] (6)

or

\[ \left( \frac{\partial P}{\partial A} \right)_V = -\left( \frac{\partial V}{\partial A} \right)_\gamma \] (7)

where the definitions in Eq. (4) are used. Equation (7) is in fact a form of the Maxwell relation of the fundamental equation (3) [11]. Following a standard thermodynamic procedure, one can easily obtain many other forms of the Maxwell relation by performing Legendre transformations on the fundamental equation (3) and using the condition of exactness. For brevity, we will not present the complete set of the Maxwell relation and only concentrate on obtaining a surface area expression by analyzing Eq. (7).

By using the properties of partial derivatives

\[ \left( \frac{\partial P}{\partial A} \right)_V = \left( \frac{\partial P}{\partial \gamma} \right)_V \left( \frac{\partial \gamma}{\partial A} \right)_V \] (8)

and

\[ \left( \frac{\partial \gamma}{\partial A} \right)_V = -\left( \frac{\partial V}{\partial A} \right)_\gamma \left( \frac{\partial \gamma}{\partial V} \right)_A \] (9)

Substitution of Eq. (9) into Eq. (8) results in
\[
\left( \frac{\partial P_v}{\partial \gamma} \right)_v - \left( \frac{\partial P_v}{\partial \gamma} \right)_v \left( \frac{\partial V}{\partial A} \right)_v \left( \frac{\partial \gamma}{\partial V} \right)_A = 1
\]  

(a)

Substitution of Eq. (10) into Eq. (7) yields

\[
\left( \frac{\partial P_v}{\partial \gamma} \right)_v \left( \frac{\partial V}{\partial A} \right)_v = 1
\]  

(b)

Rearranging Eq. (11) leads to

\[
\left( \frac{\partial P_v}{\partial \gamma} \right)_v = \left( \frac{\partial A}{\partial V} \right)_\gamma
\]  

(c)

The mathematical tools used in the above derivation from Eq. (7) to Eq. (12) are essentially equivalent to Jacobian transformations [12]

Now we may integrate Eq. (12) to find an expression for \( A = A(\gamma, V) \). Rearranging Eq. (12)

\[
dA = \left( \frac{\partial P_v}{\partial \gamma} \right)_v dV
\]  

(d)

Integrating Eq. (13) from some arbitrary state where \( A = A_0 \) and \( V = V_0 \)

\[
\int_{A_0}^{A} dA = \int_{V_0}^{V} \left( \frac{\partial P_v}{\partial \gamma} \right)_v dV
\]  

(e)

Equation (14) can be further written as
Equation (15) is an integral expression for the surface area as a function of lung volume and surface tension. This expression would, within the assumptions underlying Eq. (2), be true for any lung. The only assumption that went into its derivation is that the total distortion energy is conserved during changes in volume or area. Next, we will compare this area expression to experimental data in order to determine the constants in the equation.

**Area Calculation**

In this section, we apply Eq. (15) to both liquid-filled and air-filled lungs; the liquid-filled lung would represent the case of constant surface tension and the air-filled lung would represent the case of variable surface tension. First, we evaluate the constant parameters in Eq. (15) by comparing the integral expression with experimental data. For this purpose, it is most convenient to use experimental data of rabbit lungs since there is a wide variety of data available.

To determine the expression for

\[
\left( \frac{\partial P_y}{\partial y} \right)_y
\]

consider the data from Smith and Stamenovic [13], a linear relationship is found between surface tension \( y \) and component of lung recoil pressure \( P_y \) due to surface tension (Fig. 4 of [13]). They
reported that this same relationship is obtained for either the inflation or the deflation paths on the PV isotherm.

Smith and Stamenovic [13] did not find any systematic variations with volume, and the slope is nearly the same for any volume within the interval of 20-95% total lung capacity (TLC). This would allow us to simplify Eq. (15) as follows

\[ A = A_0 - \left( \frac{\partial P}{\partial \gamma} \right) \nu \left( \int dV = A_0 - \left( \frac{\partial P}{\partial \gamma} \right) \nu (V - V_0) \right) \]  

(16)

Figure 1 is based on the data of Smith and Stamenovic [13]. A linear least-squares fit is performed, resulting in a linear correlation coefficient of 0.988. Taking the slope of this figure, we find that

\[ \left( \frac{\partial P}{\partial \gamma} \right) \nu = 0.276 \ \text{cmH}_{2}O \ \text{mJ/m}^2 = 26993 \ \frac{1}{\text{m}} \]  

(17)

Bachofen et al. [14] normalized the above result to rabbit lungs with a TLC of 120 ml. By performing a similar linear fit to the normalized data, we obtain

\[ \left( \frac{\partial P}{\partial \gamma} \right) \nu = 0.0324 \ \frac{\text{m}^2}{\text{TLC}} \]  

(18)

Substitution of Eq (18) into Eq. (16) yields

\[ A = A_0 - 0.0324 (V - V_0) \]  

(19)
Here $A$ and $A_0$ are in m² and $V$ and $V_o$ are in %TLC (i.e., a number between 0 and 100).

To evaluate $A_0$, we must consider two cases: 1) lungs with constant surface tension and 2) air-filled lungs.

1) Lungs with constant surface tension

If lungs are filled with salt-water, the surface tension between the tissue and the fluid is equal to zero. For lungs filled with other liquids, the surface tension of the tissue-fluid interface may also be constant. For example, Smith and Stamenovic [13] observed that constant surface tension is maintained for the lungs filled with a series of different fluids.

A convenient reference point for $A_0$ and $V_o$ is 40% TLC. First, this is near the midpoint of the total lung volume. Using 0% TLC as the reference state would not be practical because it is not possible to achieve this volume under normal experimental conditions. At 100% TLC, the PV curve becomes extremely steep due to tissue effects. Second, there is a wide variety of data available for the surface area measured at 40% TLC [14-17]. Figure 2 shows experimental data obtained at 40% TLC from references [14-17]. By curve-fitting these data with the least squares procedure, we obtain that

$$A_0 = 3.03 - 0.707 \gamma^{0.366} \tag{20}$$

where $A_0$ has the unit of m² and $\gamma$ the unit of m/l/m². Substituting Eq. (20) into Eq. (19) for reference state $V_o = 40$ % TLC

$$A = 3.03 - 0.707 \gamma^{0.366} + 0.0324(V - 40)$$

i.e.,
\[ A = 0.0324V - 1.734 - 0.707\gamma^{0.366} \] (21)

where \( A \) has the unit of \( \text{m}^2 \), \( V \) the unit of percent TLC and \( \gamma \) the unit of \( \text{mJ/m}^2 \). This represents the surface area as a function of lung volume and surface tension in the case of constant surface tension.

Comparison of this model prediction Eq. (21) with experimental data is shown in Fig. 3 for saline-filled lungs [17] where surface tension \( \gamma = 0 \). The only discrepancy occurs at near 100% TLC, which corresponds to the observation of the extremely steep PV curve at 100% TLC. Figure 4 shows the model comparison with detergent rinsed lungs [15] (i.e., \( \gamma = 20 \text{ mJ/m}^2 \)). Within the error limits of the experimental data, the fit is reasonably good.

2) Air-filled lungs

In air-filled lungs, the tissue-air interface is coated by pulmonary surfactant which changes the surface tension when the surface area is varied. Therefore, the surface area of the lungs depends on not only lung volume but also surface tension.

To account for this effect, consider \( A_0 \) to be the sum of the area when \( \gamma = 0 \) and the deviation in area due to surface tension

\[ A_0 = A_0|_{\gamma = 0} - f(\gamma) \] (22)

where \( f(\gamma) \) is an expression that relates the change in surface tension to the change in area.

To evaluate \( f(\gamma) \), consider the \textit{in vitro} data on the \( \gamma \)-A response of pulmonary surfactant obtained by Schürch et al. with the captive bubble surfactometer [18,19]. Under quasi-static conditions, they demonstrated that hysteresis of a \( \gamma \)-A loop can be avoided by preventing over compression of the surfactant film. Under these circumstances, the \( \gamma \)-A response is highly linear.
Figure 5 shows such an observation for modified porcine surfactant (Curosult) and rat pulmonary surfactant [18,19]. By least squares linear regression, we find that

\[ A_1 = 6.7 \times 10^{-3} \gamma - 0.834 \]  \hspace{1cm} (23)

where \( A_1 \) is the area relative to the maximum surface area. In Eq. (23), the surface tension is decreased by compressing the captive bubble. However, if one does not control the area, the area will be determined by the action of surface tension in order to minimize the area. For two bubbles of equal volume, the one with the higher surface tension will have the smaller area. In addition, Eq. (23) has been normalized to the maximum area. To provide the correct units to insert this equation into Eq. (22), we have to dimensionalize the expression for \( f(\gamma) \). We consider the area at 100% TLC to be the maximum area. Data from Gil et al. [17] and Bachofen et al. [14-16] have an average area at 100% TLC of 3.8 m².

By incorporating the above considerations, we can obtain the expression for \( f(\gamma) \) as follows

\[ f(\gamma) = -0.025 \gamma \] \hspace{1cm} (24)

where \( f(\gamma) \) has the unit of m² and \( \gamma \) the unit of mJ/m². Substituting Eq. (24) into Eq. (22) and then into Eq. (19), the expression for air-filled lungs is

\[ A = A_0|_{\gamma = 0} - 0.025 \gamma - 0.0324(V - V_0) \]  \hspace{1cm} (25)

Since at 40% TLC the surface tension is approximately equal to zero [14-16], we can again use this as the reference condition and write down
To evaluate Eq. (26) for air-filled lungs, one requires information about the surface tension and the volume. The only available source of such data is provided by Bachofen et al. [14]. The area of the lungs was determined by evaluating light microscope data and electron microscope data; the resulting areas differ slightly because of a magnification effect. In Figs. 6 and 7, the predicted areas according to Eq. (26) are compared to the experimental data. For one and half volume cycles, the agreement is slightly better for electron microscope data (Fig. 7).

### Energy and Work Calculations

The preceding section gives the area expressions from the present lung modelling. In this section we present energy and work expressions for lungs, and again two cases are dealt with separately: 1) lungs with constant surface tension and 2) air-filled lungs. In the first part of this section we give the expressions for the distortion energy $U$, and the expressions for various work forms will be presented in the second part of this section.

**Expressions for the distortion energy**

1) Lungs with constant surface tension

First, from Eq. (21), we find an expression of surface tension $\gamma$

$$\gamma = ( - 1.41 A - 0.0457 V - 2.45 )^{2.73}$$

(27)

According to the definition Eq. (4), we can write that
\[
\int U \, dV = \int P_y \, dV - f(A) \tag{28}
\]

where \( f(A) \) is constant of integration, a function of surface area. Upon using Eq. (18), we obtain that

\[
\int P_y \, dV = \int 0.0324 \gamma \, dV
\]
\[
= \int 0.0324 \left( -1.41A - 0.0457V - 2.45 \right)^{1.73} \, dV
\]
\[
= 0.190 \left( -1.41A - 0.0457V - 2.45 \right)^{1.73} \tag{29}
\]

Substituting Eq. (29) into Eq. (28) yields

\[
U = 0.190 \left( -1.41A - 0.0457V - 2.45 \right)^{1.73} - f(A) \tag{30}
\]

Similarly, by using Eq. (27), we integrate

\[
- \int \gamma \, dA = - \int \left( -1.41A - 0.0457V - 2.45 \right)^{1.73} \, dA
\]
\[
= 0.190 \left( -1.41A - 0.0457V - 2.45 \right)^{1.73} \tag{31}
\]

And, according to the definition of the partial differenation of the distortion energy Eq. (5), we obtain that

\[
U = 0.190 \left( -1.41A - 0.0457V - 2.45 \right)^{1.73} - g(V) \tag{32}
\]

where \( g(V) \) is an integrating constant, a function of lung volume.

Comparing Eqs (30) and (32), we can finally write that

\[
U = 0.190 \left( -1.41A - 0.0457V - 2.45 \right)^{1.73} + K \tag{33}
\]

where \( K \) is a constant. Upon imposing the boundary condition for the distortion energy \( U; V = 0 \),
\[ \gamma = 0, \ A = 1.734 \ (\text{from Eq. (21)}, \ \text{and} \ U = 0, \ \text{we can find that} \ K = 0; \ \text{hence, Eq. (33) reduces to} \]

\[ U = 0.190( -1.41A - 0.0457V - 2.45)^{1.73} \tag{34} \]

where \( U \) has the unit of mJ, \( A \) the unit of m^2, and \( V \) the unit of percent TLC. It is seen that for the constant distortion energy \( U \), the relation between the area and the volume is linear. This result is shown in Fig. 8.

By combining Eqs. (27) and (34), we find that

\[ U = 0.190 \gamma^{1.56} \tag{35} \]

Equations (34) and (35) can be verified by the definitions given in Eq. (4) and Eq. (6). In addition, these equations show qualitative agreement with expected trends of \( U \) as a function of \( \gamma \): a) when \( \gamma = 0 \), the distortion energy equals zero, and b) the distortion energy increases as the surface tension increases.

2) Air-filled lungs

Again, we will integrate the partial derivatives of the distortion energy \( U \) in Eq. (4) to obtain the final expressions for the distortion energy. In general, for air-filled rabbit lungs we can write from Eq. (26) that

\[ A = 0.0324V - C_1 \gamma - C_2 \tag{36} \]

where \( C_1 \) and \( C_2 \) are positive constants that depend on the reference state. Rearranging the above equation yields
By using Eqs. (18) and (37), we find that
\[
\int P_y \, dV = \int \left( \frac{0.0324^2 V}{C_1} - \frac{0.0324 A}{C_1} - \frac{0.0324 C_2}{C_1} \right) dV
\]
\[
= \frac{0.0324^2 V^2}{2C_1} - \frac{0.0324 A V}{C_1} - \frac{0.0324 C_2 V}{C_1}
\]
(38)

By using Eq. (37), we obtain that
\[
- \int \gamma \, dA = \int \left( - \frac{0.0324 V}{C_1} - \frac{A}{C_1} - \frac{C_2}{C_1} \right) dA
\]
\[
= - \frac{0.0324 A V}{C_1} - \frac{A^2}{2C_1} - \frac{C_2 A}{C_1}
\]
(39)

Comparing Eqs. (38) and (39), we obtain that
\[
U = \frac{0.0324^2 V^2}{2C_1} - \frac{0.0324 A V}{C_1} - \frac{0.0324 C_2 V}{C_1} - \frac{A^2}{2C_1} - \frac{C_2 A}{C_1} - K
\]
\[
= \frac{0.0324^2 V^2}{2C_1} - \frac{0.0324 A V}{C_1} - \frac{A^2}{2C_1} - \frac{C_2}{C_1} \left( 0.0324 V - A \right) - K
\]
(40)

where \( K \) is a real number and can be determined by imposing the boundary condition: when \( V = 0 \), \( \gamma = 0 \) and \( A = C_2 \), the distortion energy \( U = 0 \). Therefore,
\[
K = \frac{C_2^2}{2C_1}
\]
Equation (40) is then written as

\[ U = \frac{0.0324^2 V^2}{2C_1} - \frac{0.0324 A V}{C_1} - \frac{A^2}{2C_1} - \frac{C_2}{C_1} (0.0324 V - A) - \frac{C_2^2}{C_1} \]  

(41)

For a given distortion energy, we can solve for the surface area as a function of the lung volume V

\[ A = \frac{0.0324 V - C_2 \pm \sqrt{(0.0324 V - C_2)^2 - [(0.0324 V)^2 - 2 \times 0.0324 C_2 - C_2^2 - 2 C_1 U]}}{2 \times 0.0324 V - C_2 \pm \sqrt{2 C_1 U}} \]

(42)

here \( A \) is in m\(^2\), \( V \) in percent TLC, \( C_1 = 0.025 \) and \( C_2 = 1.264 \). The graphical representation of this equation is shown in Fig. 9.

By comparing Eq. (37) and (41), we can also express \( U \) as a function of \( \gamma \)

\[ U = \frac{C_1 \gamma^2}{2} \]

(43)

Again, from this equation, \( U = 0 \) when \( \gamma = 0 \), and the distortion energy increases as the surface tension increases.

Expressions for work

In general, for a quasi-static process, we can write the mechanical work \( W \) as

\[ W = \int P \, dV \]

(44)

where \( P \) is transpulmonary pressure. In our model, we write that \( P = P_t + P_r \) with \( P_r \) being the pressure for saline-filled lungs; therefore,
\[ W = \int P_i dV - \int P_r dV \]
\[ = W_r - W_r \]

From the definition of the distortion energy Eq. (5), we obtain that

\[ \delta W_r = P_r dV = dU - \gamma dA \]

where \( \delta \) indicates a path-dependent process.

1) Lungs with constant surface tension

Since the distortion energy \( U \) is a function of the surface tension \( \gamma \) as shown in Eq. (35), under the condition of constant surface tension the distortion energy \( U \) must be constant, i.e., \( dU = 0 \). From Eq. (46), we can then obtain that

\[ \delta W_r = P_r dV = \gamma dA \]

Substituting Eq. (47) into Eq. (45), we find that

\[ W = \int P_i dV - \int \gamma dA \]

where the mechanical work \( \int P dV \) is the sum of the tissue work \( \int P_i dV \) and the surface work \( \int \gamma dA \). To evaluate these work expressions, we need to find an expression of the transpulmonary pressure \( P \). From Smith and Stamenovic [13], we can find experimental data for \( P \) as a function of the surface tension and the volume. By fitting their data within the volume range of 20-90% TLC, we obtain the following
for inflation: \[ P = 0.270 \gamma - 2.36 - 2.8 \times 10^{-5} V^{2.75} \] (49)

with correlation coefficient \( r = 0.985 \) and

for deflation: \[ P = 0.270 \gamma - 0.989 - 2.04 \times 10^{-4} V^{2.29} \] (50)

with correlation coefficient \( r = 0.997 \).

To obtain the mechanical work in unit Joule, we further convert Eq. (50) into

for inflation: \[ P = 2.63 \times 10^{4} \gamma - 230 - 5.22 \times 10^{13} V^{2.75} \] (51)

and

for deflation: \[ P = 2.63 \times 10^{4} \gamma - 96.3 - 7.19 \times 10^{11} V^{2.29} \] (52)

where \( P \) is in Pa, \( V \) in \( m^3 \), and \( \gamma \) in \( J/m^2 \).

Now, we evaluate the mechanical work for a change in volume between 20 to 100% TLC for lungs normalized as volume 120 mL. By integrating Eqs. (51) and (52) with respect to volume \( V \), we obtain that

for inflation: \[ W = 2.52 \gamma - 0.0496 \] (53)

and

for deflation: \[ W = 2.52 \gamma - 0.0367 \] (54)

where \( W \) is the unit of Joule and \( \gamma \) in the unit of \( mJ/m^2 \). The graphical representations of Eqs. (53)
and (54) are shown in Figs. 10 and 11.

By using Eq. (47), the surface work may be expressed as

\[ \int \gamma dA = \gamma \int dA = P_\gamma (V_2 - V_1) = 26300 \gamma (V_2 - V_1) \tag{55} \]

where the last equality employed the linear relationship between \( P_\gamma \) and \( \gamma \) [13]. The surface work for a change in volume between 20 to 100% TLC is plotted in Figs. 10 and 11. Also included there are the total work, which is equal to the mechanical work, and the tissue work which is the difference between the mechanical work and the surface work.

In Fig. (10), it is noted that the tissue work is constant as its expression \( \int P \, dV \) implies. The mechanical work and the tissue work increase as the surface tension increases. In Fig. 11, the values of the work are all negative since the lung volume is decreasing upon deflation.

2) Air-filled lungs

In this case, the distortion energy \( U \) is not constant as volume changes, and the change in \( U \) must be accounted for in the calculation of the work. From the definition of the distortion energy \( dU \), Eq. (5), we find that

\[ \int P \gamma dV = \int dU - \int \gamma dA \tag{56} \]

hence,

\[ \int P dV = \int P_\gamma dV - \int \gamma dA = U_2 - U_1 - \int \gamma dA - \int P_\gamma dV \tag{57} \]

Using Eq. (42) with the constant \( C_1 = 0.025 \), we reduce Eq. (57) to
Again, we use the experimental data from Smith and Stamenovic [13] to evaluate the integrals in Eq. (58). By using the trapezoid rule and a spreadsheet computer software, we obtain the tissue work \( \int P \, dV \), the surface work \( \int \gamma \, dA \), and the experimental mechanical work \( \int P \, dV \) as shown in Figs. 12 and 13 for both inflation and deflation of the air-filled lung. Further, we employ Eq. (58) to calculate the total work \( \int P \, dV \) in attempt to compare this equation to the experimental mechanical work. As shown in Figs. 12 and 13, the two are reasonably close, which validates our model expression for the distortion energy for air-filled lungs.

\[
\int P \, dV = 0.0125 (\gamma_2^2 - \gamma_1^2) - \int_1^2 \gamma \, dA - \int_1^2 P \, dV
\]  

(58)

**Relation between Surface Tension and Volume**

During the lung inflation and deflation cycle, the lung volume is often monitored. It is of interest to know the surface tension values at the mean time. For air-filled lungs, we have obtained the expression for the surface area as a function of the volume and the surface tension, Eq. (26), and hence we can predict the surface tension for a given lung volume by employing the work expression, Eq. (58). The method adopted here is an iterative one since an explicit expression of the surface tension as a function of the volume is difficult to obtain due to the integral expression of the work.

The procedure of the iteration is as follows: for a given volume \( V \) - guess a surface tension \( \gamma \) - calculate the area \( A \) - calculate \( \int \gamma \, dA \) - calculate \( \Delta U \) - calculate \( \gamma \) - iterate, until convergence occurs. In calculating \( A \), Eq. (26) is used as

21
\[ A = 0.0324V - 0.025\gamma - 1.384 \]  

where the air-filled lung volume is normalized at 120 mL and the constant is obtained through the electron microscope data of Bachofen et al. [14]. In calculating \( A \), Eq. (58) is used based on the experimental data from Smith and Stamenovic [13]. In calculating \( \gamma \), Eq. (42) is employed when \( U_2 - U_1 \) is known:

\[ \gamma_2^2 = \gamma_1^2 - \frac{2(U_2 - U_1)}{C_1} \]  

(60)

with \( C_1 = 0.025 \). Under the condition of lung inflation, we have \( U_1 = 0 \) when \( \gamma = 0 \); hence,

\[ \gamma_2 = \sqrt{\frac{2U_2}{C_1}} \]  

(61)

Under the condition of lung deflation, the zero energy state should be \( V = 20\% \) TLC, and energy decreases from \( V = 0 \) to that point; therefore, Eq. (60) has to be used in this case. Figure 14 shows the results of the iteration method, as compared to the experimental measurements of Smith and Stamenovic [13]. Two tolerances in surface tension are used: 0.01 and 0.05 mJ/m². It can be seen that the model prediction of the surface tension is in agreement with the experimental values within the error limits.

**Discussion**
From a thermodynamic point of view, we obtain a general expression of the distortion energy, Eq. (5). From a Maxwell relation for this energy expression, we obtain a relation between the recoil pressure and the surface tension, Eq. (7). By using the properties of partial derivatives, we convert Eq. (7) into Eq. (12). Based on Eq. (12), we derive the expression of the surface area, Eq. (15).

For the two separate cases: 1) lungs with constant surface tension and 2) air-filled lungs, we provide explicit expressions for the surface area, the distortion energy and mechanical work by obtaining the fitting parameters upon comparing the model to the experimental data. The model prediction is in good agreement with the experiment in all situations. In finding the relation between the surface tension and the volume, an iteration method is adopted in order to compare the model prediction and the experiment. Again, the agreement is good.

The distinction between the two cases is necessary since the surface tension effect in the air-filled lungs differs from that in the lungs with constant surface tension. For the air-filled lungs, the surface tension will increase when the lung volume increases, and this will affect the distortion energy in two ways: a) the work associated with the surface area increase will increase as the volume increases, compared with the situation in the lungs with constant surface tension, and b) the changed surface tension will distort the tissue geometry and hence change the energy stored in the tissue. As a result, the model expressions for the two cases are quite different.

The assumption of the current thermodynamic model is that the lungs under consideration keep constant temperature and no chemical component transfer exists within the system. This is equivalent to the free energy formulation of thermodynamics, i.e., choosing the grand canonical potential as the thermodynamic potential [11]. In this sense, one can reformulate the above
thermodynamic analysis with the grand canonical free energy while still obtaining all the results relating to the mechanical work of the lung movement.

The second assumption of the model is that the processes analyzed are supposed to be quasi-static, and this will require that the hysteresis is avoided by preventing overcompression of the alveolar surfactant film [18,19]. This assumption is also equivalent to the one that the distortion energy of the lungs is conserved during the change in lung volume or surface area; as a consequence, the distortion energy is expressed as a function of the volume and the area. Physically, this second assumption means that the local alveolar mechanical balance is always reached at a much faster rate than the breathing process.

Similar to the Wilson model [8], the current model does not rest on any assumption of the alveolar geometry and mechanical structure of the parenchyma; hence, the results provide no information about the details of parenchyma geometry and structure. To incorporate this information, a detailed model including alveolar geometrical parameters, which impose additional constraints, would be needed.
References


Lung Surface Area (m²) vs. Surface Tension (mJ/m²)

Experiment

\[ A = 3.03 - 0.707\gamma^{0.366} \]
Experiment Modei Prediction

Lung Volume (% TLC)

Lung Surface Area (m²)

- Experiment
- Model Prediction
Experiment - Model Prediction

Lung Surface Area (m²) vs Lung Volume (% TLC)

- ○ Experiment
- - Model Prediction
Rat LES (compression)

Rat LES (expansion)

Pig LES

Pig LES

Pig LES [50 μg/ml] (compression)

Pig LES [50 μg/ml] (expansion)

Pig LES [1 mg/ml] (compression)

Pig LES [1 mg/ml] (expansion)
Lung Volume (% TLC)

Lung Surface Area (m²)

○ Experiment

- Model Prediction
Experiment 4 Model Prediction

Lung Volume (% TLC)

Lung Surface Area (m²)

○ Experiment

- Model Prediction

Lung Volume (% TLC)
Constant Surface Tension

Contours of Energy - /lung.model/calcul/uconstg.grf

Lung Surface Area (m²)

Lung Volume (% TLC)

U = 0
U = 10
U = 20
U = 30
Lung Inflation

Work (J)

Volume (% TLC)

- Tissue Work
- Surface Work
- Change in Distortion Energy
- Total Work
- Mechanical Work
Lung Deflation

/lung.model/calcwork/work.def.grf

- Work (J)
- Volume (% TLC)

- ○ Tissue Work
- □ Surface Work
- ◊ Change in Distortion Energy
- ▽ Total Work
- * Mechanical Work

- 0.00
- -0.02
- -0.04
- -0.06
- -0.08
- -0.10

10 20 30 40 50 60 70 80 90 100

[F. 13]
Rabbit Lungs

/lung.model/calcgam/vgam0.0385.grf

- Experiment (Smith & Stamenovic)
- Model (Tolerance=0.01)
- Model (Tolerance=0.05)

Lung Volume (% TLC) vs. Surface Tension (mJ/m²)
Ph.D. Related

Publication #9

(in preparation)
An Investigation of the Surface Tension - Surface Area Hysteresis, the Maximum Surface Tension, and the "Squeeze-Out" Plateau at Two Concentrations of Bovine Pulmonary Surfactant

R.M. Prokop, A. Jyoti, M. Eslamian, M. Mihaila, Z. Policova and A.W. Neumann

By using the Axisymmetric Drop Shape Analysis - Captive Bubble methodology, two concentrations of bovine pulmonary surfactant were investigated. The amount of hysteresis observed in the surface tension - surface area (\(\gamma-A\)) isotherm varied with the degree of interfacial compression. This observation was more apparent for the lower surfactant concentration. In this case, surfactant removed from the interface during previous compressions was slower to return to the surface. This phenomenon also influenced the maximum surface tension (\(\gamma_{max}\)) achieved upon expansion of the interface: the lower concentration surfactant resulted in a higher \(\gamma_{max}\) as surfactant was absent from the surface due to slow adsorption. The so-called "squeeze-out" shoulder was closely examined, and it was found that this shoulder was clearly present throughout the experiment at low surfactant concentrations. At the higher concentration, this shoulder would dampen out with progressive cycling. This observation can also be explained by the slow adsorption of surfactant to the interface in the low concentration experiments.

(To be submitted to Journal of Applied Physiology)
Ph.D. Related

Publication #10

(in preparation)
The ADSA Technique and Its Applications


This chapter includes an introduction of the Axisymmetric Drop Shape Analysis (ADSA) as a powerful technique in interfacial tension and contact angle measurements. The theoretical basis and numerical algorithm used in ADSA are presented. As examples of applications in interfacial tension measurements, the following are presented: 1) interfacial tensions of pure liquids and solutions, where the superior accuracy of ADSA is illustrated; 2) temperature dependence of interfacial tensions, in which surface entropy information is obtained; 3) pressure dependence of interfacial tensions, from which the thickness of the interface (Gibbs dividing surface) is calculated; 4) ADSA used as a film balance, which shows many advantages over the conventional Langmuir balance; 5) dynamic interfacial tension measurements, where versatility of ADSA is shown; 6) static and dynamic interfacial tensions of protein solutions, showing ADSA applied to studies of biological interfaces; 7) lung surfactant surface tensions, where low surface tensions (<1 mJ/m²) are measured; 8) lipid surface tension measurements.

In contact angle measurements, the following are presented: 1) again, the superior accuracy of ADSA; 2) advancing, receding contact angles and contact angle hysteresis; 3) line tension detection by the drop-size dependence of contact angle measurement; 4) validation of the equation of state approach by contact angle and surface tension measurements for various polar and non-polar liquids; 5) contact angles on self-assembled-monolayers.

Ph.D. Related

Publication #11

(in preparation)
Dynamic Surface Tension of Protein and Protein/Small Organic Molecule Mixtures

P. Chen, R.M. Prokop and A.W. Neumann

This chapter describes the interfacial behaviour of protein solutions. The topics cover: 1) temperature dependence of interfacial tension of albumin solution, in which criteria of finding equilibrium interfacial tensions are presented; 2) concentration dependence of interfacial tension of albumin solutions, where negative spreading pressures and depletion of protein molecules at the interface are analysed; 3) dynamic surface tension of protein/small or medium-sized organic molecule mixtures, in which competitive adsorption to the interface, stability of surface adsorption of molecules, and the squeeze-out mechanism of small molecules are presented.

Ph.D. Related

Conference Papers

#1, 2, 3, 4
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Supported by a grant from the Henkel Corporation
BLOCK COPOLYMER ADSORPTION AT THE TOLUENE-WATER INTERFACE

R. M. Prokop1, M. L. Hair2, A. W. Neumann2

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The adsorption of polymers at the interface between organic solvents and water is of interest in many applications ranging from emulsion polymerization to oil extraction. Determining the role of the polymer at the interface is not simple. Recently we have applied the ADSA (pendant drop) technique to this problem. A pendant drop of water is immersed in toluene and a block copolymer of polyethylene oxide-b-PS is adsorbed at the interface from the toluene solution. Both blocks of this polymer are soluble in toluene but the polystyrene is insoluble in water and forms a depletion layer at the water/organic interface. With the polymer used in these studies, the changes in surface tension during adsorption and then surface compression can be seen as four distinct phases. Initially there is slow diffusion of the polymer to the interface and equilibrium is not achieved until at least six hours have elapsed. When the equilibrated polymer layer is compressed by reducing the size of the drop three further phases can be distinguished:

i) There is a slow decrease in surface tension as the area per molecule is decreased

ii) This is followed by a rapid change in surface tension with area

iii) In the final stage the surface tension changes dramatically with small changes in area.

These changes are interpreted in terms of the relative sizes of the two components of the block copolymer.

Contact Angle Measurements on Co-Polymer Surfaces by Axisymmetric Drop Shape Analysis: Testing of an Equation of State Relation

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Contact angles of various polar and non-polar liquids on two different co-polymer (polar) surfaces were measured by Axisymmetric Drop Shape Analysis. It was found that the resulting contact angles for some liquids are complicated. Using Axisymmetric Drop Shape Analysis, various complexities, such as slip and stick of the three-phase contact line and chemical non-linearity of the solid surfaces, will be illustrated. All of these effects affect the contact angles in an unpredictable manner and, therefore, these angles should not be used to interpret surface energetics. If one omits such inconclusive contact angle data, the usual well-known smooth curves in the $\gamma_{lv} \cos \theta$ versus $\gamma_{lv}$ plots are obtained, just as for non-polar surfaces. Essentially constant solid-vapour surface tensions are obtained from the equation of state approach for interfacial tensions.
Static and Low Rate Dynamic Contact Angles: Measurement and Interpretation

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Axisymmetric Drop Shape Analysis is a versatile surface tension and contact angle technique, applicable to both pendant and sessile drops. The advantage of using ADSA for sessile drop contact angle measurements is that it yields high accuracy and consistency. Using this technique for static and dynamic contact angle measurements, quantitative measure of the solid-liquid system can be obtained; examples will be given for a non-polar FC-722 surface and three co-polymer (polar) surfaces. It was found that low rate dynamic contact angles are essentially identical with static angles up to a rate of 0.7 mm/minute for the three-phase contact line. It was also found that the contact angle phenomena on these polar surfaces are complicated. Such complexities affect the contact angle interpretation of surface energetics.

Axisymmetric Drop Shape Analysis: Its Scope for Measuring Interfacial Tensions and Contact Angles


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Axisymmetric Drop Shape Analysis (ADSA) is a versatile surface tension and contact angle technique, applicable to both pendant and sessile drops. In the past, ADSA has been used in a variety of situations for surface tension and contact angle measurements. The ADSA illustration given here will include the pressure dependence of interfacial tensions at liquid/liquid interfaces, film balance experiments with insoluble films, dynamic surface tension measurements, the time dependence of liquid/liquid interfacial tensions in the presence of surface active materials, ultralow interfacial tensions, drop size dependence of contact angles and line tensions, and contact angle measurements with an accuracy exceeding other methods by an order of magnitude.

The Compression Rate Dependence of a DPPC Isotherm

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Using Axisymmetric Drop Shape Analysis (ADSA) as a film balance, the surface characteristics of a dipalmitoyl phosphatidylcholine monolayer at the air/water interface were investigated. It was found that the rate of compression of the monolayer had no effect on the shape of the surface pressure - surface area isotherm. This study was motivated by a recent ADSA investigation which showed a significant dependence of the compression rate on another monolayer type, namely oxaedecanol. The difference is explained by considering molecular structure, the molecular pattern upon monolayer formation, and the malleable nature of the DPPC domains.
Determination of the Interfacial Tension of Sessile Drops of Arbitrary Contact Angle from the Drop Height and Diameter

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A method has been developed to calculate the interfacial tension of a sessile drop by measuring the drop height and diameter. This method is an improvement over existing height-diameter methods because it is not restricted to a contact angle of 180°. Although this procedure works in theory for a sessile drop with any contact angle, practical limitations reduce the range of angles to roughly 50°-180° (with a maximum error in interfacial tension of 5%) but do not restrict the range of interfacial tensions (at least 0.01 mJ/m² to 72 mJ/m²). This method is particularly useful for measuring ultralow interfacial tensions since it is more easily applied than the spinning drop method.

SPREADING OF WATER BASED GRAVURE INK ON PAPER, R. Auerbach, J.N. Hruszewicz, E. Matijevic and R. Sprycha, CAMP Clarkson University, Potsdam, NY 13699; Sun Chemical Corporation, Carlstadt, NJ 07072

Surface energy of coated paper and paper coating binder film was determined via contact angle measurements of water, diiodomethane and glycerol. Kinetics of spreading of water based gravure inks on the coated paper as well as paper coating binder film was measured by means of dynamic contact angle measurements and high speed digital imaging system. Viscosity of gravure ink at different stages of drying was also measured using controlled rate rheometer. Interactions between ink and paper were found to affect significantly the process of spreading of ink on the paper surface. Sorption of water by substrate results in a rapid increase of ink viscosity at the interfacial region. Acid-base reactions are responsible for destabilization of the ink at the interface resulting in pigment and acrylic resin flocculation and/or coagulation. All the above slow down the ink spreading process and can have detrimental effect on the aesthetic appearance of the print.

CORRESPONDING STATES CORRELATIONS FOR THE SURFACE TENSION OF MOLTEN ALKALI METALS, Mohammad H. Ghatee and Ali Boushehri.
Department of Chemistry, Shiraz University, Shiraz, 71454, Iran

According to the phenomenological scaling and consideration of the law of corresponding states, two sets of reduced coordinates, \( \sigma_1 - T_{1*} \) (I) and \( \sigma_2 - T_{2*} \) (II), for the prediction of surface tension of alkali metals are introduced. In both of the correlations, the temperature of melting is used as the corresponding temperature for the different alkalics, resulting in a promising correlations. The relation (II) in which \( T_{2*} \) is scaled distance from \( T_b \) is applied accurately to molten K, Rb, and Cs and with less accuracy to Li and Na over an extensive range of temperature. Quantum effects are considered to be responsible for this. On the other hand, the relationship (I) in which \( T_{1*} \) is scaled distance from \( T_c \) the critical temperature, is applied quite well and such a distinction with the predicted accuracy among the metals is not clearly observed. The relation (I) predicts the surface tension of molten alkalis with maximum average deviation of 1.29%, while the relationship (II) has the advantage that does not need any critical parameter and still predict the surface tension of the metals accurately, with maximum average deviation of 2.43%. Relation (I) for its accuracy and relation (II) for both its accuracy and practical applicability are advantageous over the literature counterpart.
Ph.D. Related

Conference Papers

#5, 6, 7
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Salt Lake City, Utah, U.S.A.

June 11-14, 1995
higher polymer concentrations, the capillary mode evolves into a Rayleigh mode. In addition, a second mode appears in the SLLS power spectra at these higher polymer concentrations. The crossover phenomenon and appearance of the second mode are molecular weight dependent. Results illustrating these observations will be shown, and then discussed within the existing framework of theory. The importance of the data analysis procedure will be emphasized. Finally, SLLS data from a common household product illustrating this phenomenon will be presented.

42. A MODIFIED PLATE METHOD FOR SIMULTANEOUS CONTACT ANGLE AND SURFACE TENSION MEASUREMENTS

R Tsay, S. C. Yan, Inst. of Biomed. Eng., National Yang Ming University, Taipei Taiwan, R.O.C.

Traditional Wilhelmy plate method assumes zero contact angle for the surface tension measurements. This has been the major cause of error especially for monitoring the surface pressure of a spread monolayer under dynamic compression/expansion tests. In this research, a modified plate method was developed to avoid this problem. In this method, an image digitization technique was first applied to allocate the air-water interface adjacent to a flat plate. The surface tension and contact angle were then obtained by fitting the data points of the interface with a theoretical equation derived from Young-Laplace equation. Experiments have been done for monolayers of DPPC, DPPG, DMPC, PA and DPPC/DPPG molecules. It is found that the advancing contact angles between a sandblasted platinum plate and the water interface covered with various monolayers are ranging from 0 to 25 degree while the receding contact angles are all zero.

43. AXISSYMMETRIC DROP SHAPE ANALYSIS: ITS SCOPE FOR MEASURING INTERFACIAL TENSIONS AND CONTACT ANGLES

D.Y. Kwok, O del. Rio, S.S. Susnar, A. Iyoti, R. Prokop and A.W. Neumann, Department of Mechanical Engineering, University of Toronto, Toronto, Ont., Canada M5S 1A4

Axisymmetric Drop Shape Analysis (ADSA) is a versatile surface tension and contact angle technique, applicable to both pendant and sessile drops. In the past, ADSA has been used in a variety of situations for surface tension and contact angle measurements. The ADSA illustration given here will include the pressure dependence of interfacial tensions at liquid/liquid interfaces, film balance experiments with insoluble and soluble films, dynamic surface tension measurements, the time dependence of the liquid/fluid interfacial tensions in the presence of surface active materials, ultra-low interfacial tensions, drop size dependence of contact angles and line tension, and contact angle measurements with an accuracy exceeding other methods by an order of magnitude.

44. A MICRO-DEFORMATION INSTRUMENT FOR SOFT SPHERICAL PARTICLES

D.C. Andrei, B.J. Briscoe, P.F. Luckham and D.R. Williams, Department of Chemical Engineering, Imperial College, Prince Consort Road, London SW7 2BY, UK.

The investigation of the micro-mechanical deformation of single viscoelastic particles may provide a basis for interpreting the complex rheology of concentrated dispersions of these particles in different media. It also provides an insight into the intrinsic properties of the materials to be studied. This talk will present a novel micro-deformation instrument developed for characterizing single soft particles. The deformation is imposed on the particles by compression between rigid parallel plates. The instrument has a limiting force resolution of $10^4$ N and a maximum force capability of $10^5$ N. The spatial resolution of the imposed compressive deformation is better than 0.1 μm. The two operating modes of the instrument can provide loading-unloading and stress-relaxation curves. An application of the experimental technique to viscoelastic individual particles belonging to the class of highly swollen hydrogels ('Sephadex' beads supplied by Pharmacia) will be discussed and evaluated.
102. PROTEIN ADSORPTION ON POLYMERIZED LANGMUIR-BLODGGETT FILMS OF 10, 12-PENTACOSADIYNOIC ACID

Willem Norde, Marcel Ciesbers and He Pingzheng, Department of Physical and Colloid Chemistry, Wageningen Agricultural University, P.O. Box 8038, 6700 EK Wageningen, The Netherlands.

We have constructed (multi)layers of 10, 12-pentacosadiynoic acid, \( CH_{12}(CH_2)_{11}C=CC=C(CH_2)_2COOH \), by applying the Langmuir-Blodgett (LB) technique. These films are stabilized by cross-linking, using UV radiation. The LB-films are characterized in terms of hydrophobicity and electrochemical properties. They may serve as models in studying the interaction between proteins and biological surfaces. The proteins used in this study are lysozyme, \( \alpha \)-lactalbumin and superoxide-dismutase. The proteins have similar size and shape, but they differ with respect to their isoelectric points and structural stabilities. The adsorption process was monitored by reflectometry. The experimental data provide insight in the mechanism of protein adsorption on biological membranes like surfaces.

103. THE EFFECT OF COMPRESSION RATE ON THE COLLAPSE PRESSURE OF A DPPC MONOLAYER

A. Jyoti1,2, R.M. Prokop1, D.Y. Kwok1 and A.W. Neumann1,2, Department of Mechanical Engineering, University of Toronto, Toronto, ON M5S 1A4, 2Institute of Biomedical Engineering, University of Toronto, Toronto, ON M5S 1A4, Canada.

Using a novel methodology, Axisymmetric Drop Shape Analysis, we investigated the effects of compression rate on the collapse pressure of a dipalmitoyl phosphatidylcholine (DPPC) monolayer. The rate of compression of a monolayer can be adjusted by controlling either the initial surface area per molecule of surfactant in the film, or the speed at which this area is reduced. By independently varying these parameters it was found that weak effects, if any, were elicited. This study was conducted following recent findings involving an octadecanol monolayer, which suggested that the collapse pressure is dependent upon the initial concentration of surfactant, but is invariant with the speed of film compression. Our findings may be rationalized by the rate-dependent size of "islands" of surfactant molecules as shown by epifluorescence microscopy.

104. INTERACTION OF FIREFLY LUCIFERASE WITH PHOSPHOLIPID MEMBRANES

Chung-Yih Wang and Joseph D. Andrade, University of Utah, 2480 MEB, Salt Lake City, UT 84112

Firefly luciferase has been shown to contain a carbonyl-terminal peroxisomal targeting signal (1). It is believed that luciferase is transported to lipid membranes after translation. To examine the luciferase-lipid interaction, we have prepared different liposomes by sonication. The activity of luciferase was measured after incubation with liposomes. The results suggest that phospholipids with neutral polar head groups can slightly enhance luciferase activity. However, phospholipids with net negative charge inactivate luciferase. Differential scanning calorimetry was performed with pure luciferase, with liposomes, and with the mixtures. The results show that an exothermic peak appears before the phase transition temperature of dipalmitoylphosphatidylcholine (DPPC) suggesting that the interaction of luciferase with DPPC may provide an environment which enhances the aggregation of luciferase.

105. **LANGMUIR BLODGETT STUDIES OF MODEL PROTEINS PRE-TREATED IN UREA SOLUTIONS**

D. J. Min, C. Y. Wang, and I. D. Andrade: 'Department of Materials Science and Engineering and 'Department of Bioengineering, University of Utah, Salt Lake City, UT 84112

The correlation between protein characteristics, including hydrophobicity and flexibility, and interfacial activity of protein was studied by (surface pressure)-A(interfacial area) techniques. We selected lysozyme and myoglobin as model globular proteins and B-casein as a model random-coil type protein. The interfacial activity is in the order: B-casein > myoglobin > lysozyme. For the globular proteins, the interfacial activity was enhanced by solution pretreatment (lysozyme in 8M urea and 0.2M DTT and myoglobin in 8M urea). The unfolding of globular proteins at the air/solution interface is apparently restricted by both disulfide and noncovalent bonds. The unfolding rate is mainly influenced by the disulfide bonds. The unfolding and orientation of random coil type protein, B-casein, at the interface was not influenced by the pretreatment (8M urea), as expected, probably because it is already completely unfolded at the air/solution interface.

106. **THE BEHAVIOR OF SOYBEAN OLEOSINS AND MANDUCA SEXTA APOLIPOPHORIN III PROTEINS AT THE CYCLOHEXANE/WATER INTERFACE**

Monica Tisack, Robert Lochhead, Charles McCormick, and Gordon Cannon, University of Southern Mississippi, Dept. of Polymer Science, Hattiesburg, MS 39406

Soybean Oleosins and Manduca Sexta Apolipoprophin III are proteins in plants and insects, respectively. These proteins naturally interact at oil/aqueous interfaces, and function to sequester tri- and diglycerides for energy storage. Tension measurements have been taken at the cyclohexane/water interface using the DeNouy ring method on a Kruss K12 Tensiometer and compared with phase diagram data for the former proteins. Droplet sizes for cyclohexane/water emulsions and adsorption amounts on hydrophobic spheres have also been evaluated. These proteins have also been compared with the synthetic emulsifier, hydrophobically modified poly(acrylic acid), and also with commercially used protein emulsifiers such as casein and bovine albumin.

107. **THE DYNAMIC SURFACE TENSION OF PULMONARY SURFACTANT FROM LAVAGED RABBITS**

R.M. Prokop*, A. Iyot, H. Fmdova**, C. Bryan*, P. Cox**, and A.W. Neumann**, *Dept. of Mechanical Engineering, University of Toronto, Toronto, ON M5S 1A4. **Dept. of Critical Care, Hospital for Sick Children, Toronto, ON M5G 1X8, Canada

Since its discovery in the mid-1950's, a deficiency of pulmonary surfactant has been linked to the development of Respiratory Distress Syndrome in premature neonates and in adults. The study of the surface properties of pulmonary surfactants is of interest since they are complex, multicomponent systems in which the dynamic rather than the equilibrium surface behaviour is of primary importance. We have adapted our methodology, Axisymmetric Drop Shape Analysis, to examine the dynamic properties of lung surfactant samples obtained from lavaged rabbits. The experiment conditions were systematically changed to determine which variables have the most significant effect on the surface properties. In addition, the "squeeze-out" hypothesis of soluble proteins from the interface during dynamic compression will be discussed.

108. **ACTIVITIES OF THE TRYPsin ADSORBED ON THE MODIFIED SURFACES OF LATEX PARTICLES**

Takaaki Arai and Hironobu Tsubura, Department of Industrial Chemistry, College of Industrial Technology, Nihon University, Izuoi-cho, Narashino-shi, Chiba 257, Japan

The protease trypsin was immobilized on the polymer latex particles covered with phospholipid bilayer. Influences of the lipid-coverage on the particle surfaces on hydrolysis and peptide synthesis of the immobilized trypsin were
Ph.D. Related

Conference Paper #8

(there was no abstract)
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SURFACE TENSION AND THE LUNG

Thursday, April 6, 1995
Meakins-Christie Laboratories

9:00-9:30  Dr. David Eidelman
"Introduction"

9:30-10:00  Dr. Malcolm King
"Airway surface rheology, mucociliation and clearance"

10:00-10:30  Dr. Jason Bates
"Bronchial collapse"

10:30-11:00  Dr. Mara Ludwig
"The contribution of surface forces to lung tissue hysteresis"

11:00-11:15  Break

11:15-11:45  Dr. Sam Schürch
"The surface associated surfactant reservoir"

11:45-12:15  Mr. Rob Prokop
"Measurement of lung surfactant using axis symmetric drop shape analysis"

12:15-1:00  Lunch

1:00-1:30  Dr. Yvon Cormier
"Role of surfactant in hypersensitivity pneumonitis"

1:30-2:00  Dr. Olivier Lesur
"Surfactant associated proteins in inflammatory lung disease"

2:00-2:30  Dr. Neil Sweezey
"Regulation of glucocorticoid receptors in developing lung"

2:30-3:00  Dr. Faygie Kaplan
"Identification of genes involved in the adaptation of the developing lung to the air environment"

3:00-3:30  Dr. Yves Berthiaume
"ANF and surfactant production"