REPORT DOCUMENTATION PAGE

Form Approved
OMB NO. 0704-0188

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	04/2000-03/2001	Final Report, 04/2000-03/2001 01 Apr 00 - 31 Mar 01

4. TITLE AND SUBTITLE
Acquisition of epifluorescence film balancing system

5. FUNDING NUMBERS
DAAD19-00-1-0023

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U. S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSORING / MONITORING AGENCY REPORT NUMBER
~~DAAG559710160~~
40855.1-CH-RIP

11. SUPPLEMENTARY NOTES
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13. ABSTRACT (Maximum 200 words)

Funding was given to purchase a new epifluorescence film balancing system. Request for this grant was made justifying its need for the ongoing research projects.
Different components of the system were acquired from different sources and were assembled into the system that can record fluorescence images at the air-water interface. The system was tested for its performance on the monolayers of a new amphiphilic coumarin dye. Excellent fluorescent images were recorded on the Langmuir films of a new amphiphilic coumarin dye. These images recorded at different surface pressures are presented in this report.
Further, this system is being applied to characterize the monolayers of different enzymes that interact with organophosphorus compounds at the air/water interface.

14. SUBJECT TERMS
Organophosphorus compounds, acetylcholinesterase, monolayers, biosensor, organophosphorus acid hydrolase, Langmuir films, Epifluorescence

15. NUMBER OF PAGES
8

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION ON THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

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DAAD19-00-1-0023

Acquisition of epifluorescence film balancing system

Final Progress Report
(April 2000-March 2001)

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Table of contents

(1) Statement of the problem:

This research proposal was funded for the acquisition of an epi-fluorescence film balance system. Epi-fluorescence microscopy is an important optical microscopic technique for surface chemistry study, especially for Langmuir monolayer at the air-water interface. It is planned to use this technique for characterizing the monolayers of acetylcholinesterase, organophosphorus acid anhydrase and organophosphorus acid hydrolase, which are important enzymes in the detection and decontamination of organophosphorus compounds.

(2) Summary of the most important results:

This research proposal was submitted for funding to purchase an epi-fluorescence film balance system. Epi-fluorescence microscopy is an important optical microscopic technique for surface chemistry study, especially for Langmuir monolayer at the air-water interface. By mounting a Langmuir trough on top of an inverted fluorescence microscope, fluorescence from Langmuir films can be obtained through the quartz window on the bottom of the trough. By using fluorescence probe labeling, domain behaviors of Langmuir monolayer can be visualized. Reactions and specific bindings at the air/water interface can also be monitored.

The epi-fluorescence microscopy film balance system consists of the following components (Figure 1):

1. A mini-Langmuir-trough and control system,
2. An inverted fluorescence microscope,
3. A highly sensitive video camera which can record the low fluorescence emission image of the monolayer,
4. An image processing system with real time video capture to digitize and analyze images,
5. A fast personal computer for image processing and trough control.

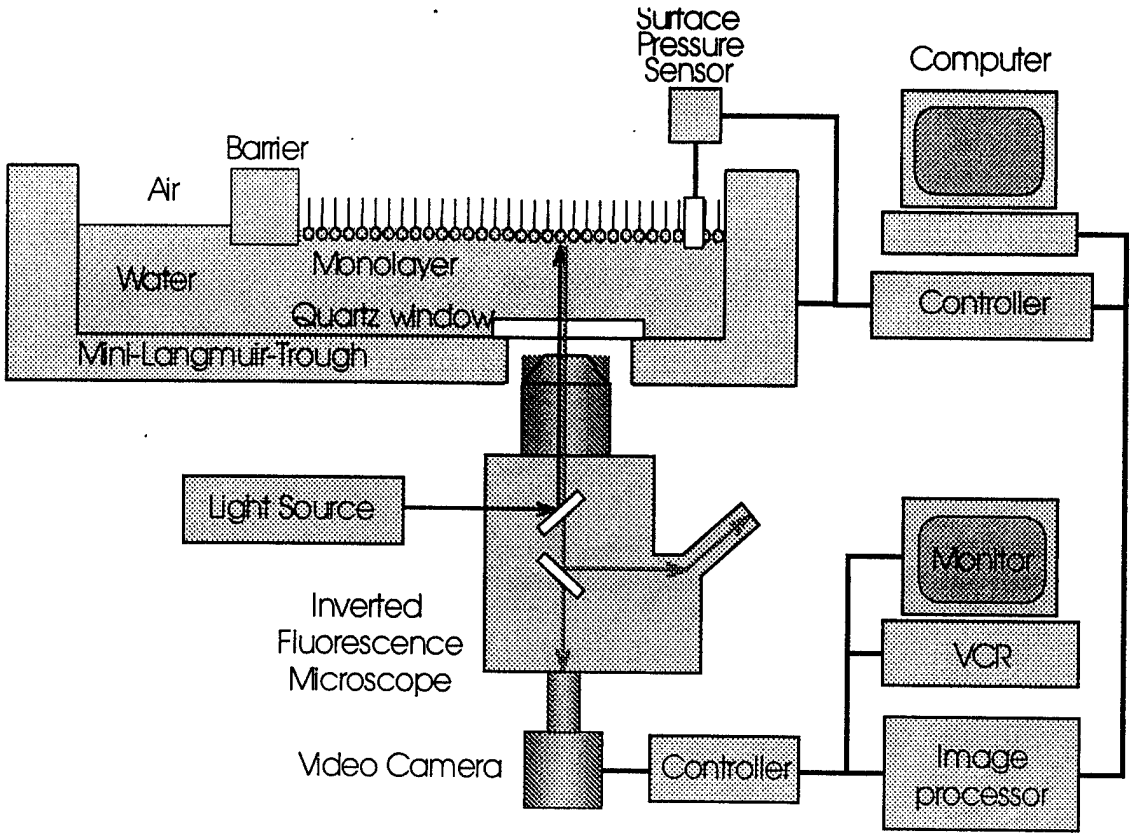

Figure 1. Diagram of the proposed epi-fluorescence microscopy system.

(a) Components of the system

The following components were purchased and the above designed set up was assembled.

i) Mini Langmuir-trough from Kibron (MicroTrough S), which has a small subphase volume suitable for our study.

ii) Olympus IX70 Fluorescence Inverted Microscope, including sets of filters and dichroic mirrors suitable for imaging two different fluorophores, and Long Working Distance Objectives.

iii) and iv) Hamamatsu Cooperation's C2400-97E, Intensified CCD (ICCD) video camera as image processing system (C5510 ARGUS 20).

v) Dell Pentium® computer was chosen for the image process and trough control.

A graduate student (Peter Kele) and a Research Assistant (Jhony Orbulescu), who were involved in research projects on characterization of Langmuir and Langmuir-Blodgett films helped in procuring these items and assembled them into a system capable of recording

fluorescent images on Langmuir films. The assembled epi-fluorescence microscopy film balance system was installed in the state-of-the-art clean room (class 1000). This room provides dust free, temperature and humidity controlled operation conditions, which are a requirement for the system. The room is well maintained by the Department of Chemistry at the University of Miami. The surface chemistry lab is under the responsibility of Dr. R. M. Leblanc.

Information on the following projects was collected using epifluorescence film balancing system.

b) Surface chemistry of enzymes for detection and decontamination of organophosphorus compounds (DAAD19-00-1-0138)

Enzymes such as acetylcholinesterase (AChE), organophosporus acid anhydrase (OPAA) and organophosphorus acid hydrolase (OPH) are important in the detection and in decontamination of organophosphorous compounds (OP). AChE is very sensitive to OP compounds and the inhibition of this enzyme is used to detect and measure the levels of OP compounds. The OPAA and OPH catalyze the hydrolysis of OP compounds. An understanding of the molecular interaction between the enzymes and OP compounds in monolayers is highly useful in designing new detection and decontaminant systems.

We are investigating the Langmuir and Langmuir-Blodgett films of these enzymes with an objective of developing a suitable and sensitive biosensor for detection of OP compounds and to develop a suitable decontaminant system.

Monolayers can be characterized by following the i) thermodynamic, ii) spectroscopic, iii) microscopic and iv) optical properties.

The thermodynamic properties can be followed by studying the surface pressure-area (π-A) and surface potential – area of pure enzyme monolayers at the interface. These thermodynamic properties suggest the molecular area and orientation of the molecules.

The spectroscopic properties can be followed by studying the absorption and fluorescence spectroscopic properties of the monolayers. We have a lab-built trough equipped with absorption and fluorescence spectrometers for recording the spectra at the interface. Spectroscopic properties indicate the aggregation state of molecules and interactions with other compounds in the films. Fluorescence microscopy and Scanning probe microscopy are being used for characterizing the films. The microscopic images present the topography of the films.

Epifluorescence images were recorded on monolayers of fluoroprobe labeled AChE at the interface. The main objective was to demonstrate the uniformity of the labeled AChE monolayer and transfer it on to an optical fiber for designing the biosensor for detection of OP compounds.

Further work is in progress with AChE and we are also working on the characteristics of the monolayers of labeled OPAA and OPH.

c) Characterization of the Langmuir films of a new amphiphilic coumarin dye, 7-aminocoumarin-4-acetic acid octadecylamide

Fluorescence spectroscopy and fluorescence microscopy are very useful in observing the assembling process of the components in Langmuir monolayers, measuring lipid diffusion and investigating molecular organization at the interface. Non-amphiphilic coumarin derivatives are widely used as blue fluorophores and their optical and photophysical properties in solution have been extensively studied. However, amphiphilic coumarin derivatives as fluorescent probes in monolayer studies have been neglected.

Langmuir monolayers and Langmuir-Blodgett (LB) films of amphiphilic dyes may have substantially different optical properties, therefore it is necessary to characterize them from this point of view. The photophysical properties of these molecules can be modified due to: (i) molecules assembled into an ordered film, (ii) polarity of the surrounding medium, (iii) structural changes caused by the applied external pressure during compression, (iv) formation of aggregates. These effects can be seen as a change in the position and/or the intensity of the bands in the UV-Vis and fluorescence spectra.

In this project, we synthesized and characterized the monolayer behavior (surface pressure and surface potential) of a new amphiphilic coumarin dye, 7-aminocoumarin-4-acetic acid octadecylamide (ACO), at the air-water interface. The spectroscopic characteristics (UV-Vis, fluorescence and fluorescence imaging) of pure and mixed films (1:20) (ACO:stearic acid and ACO:oleic acid) at the air-water interface as well as Langmuir-Blodgett films were investigated and compared with the spectroscopic characteristics of ACO in solution. These experiments provided evidence for aggregate formations during compression of the monolayer at the air-water interface. Surface pressure dependent *in situ* fluorescence imaging confirms that the fluorescent quenching of the dye with increasing surface pressure originates as a result of formation of non-fluorescent aggregates (Figure 2). Atomic force microscopy imaging of a pure ACO Langmuir-Blodgett film shows that the size of these aggregates is in the nanometer scale. This work provides information that ACO forms a stable monolayer and may be utilized as an efficient molecular probe for monolayer studies.

Figure 2

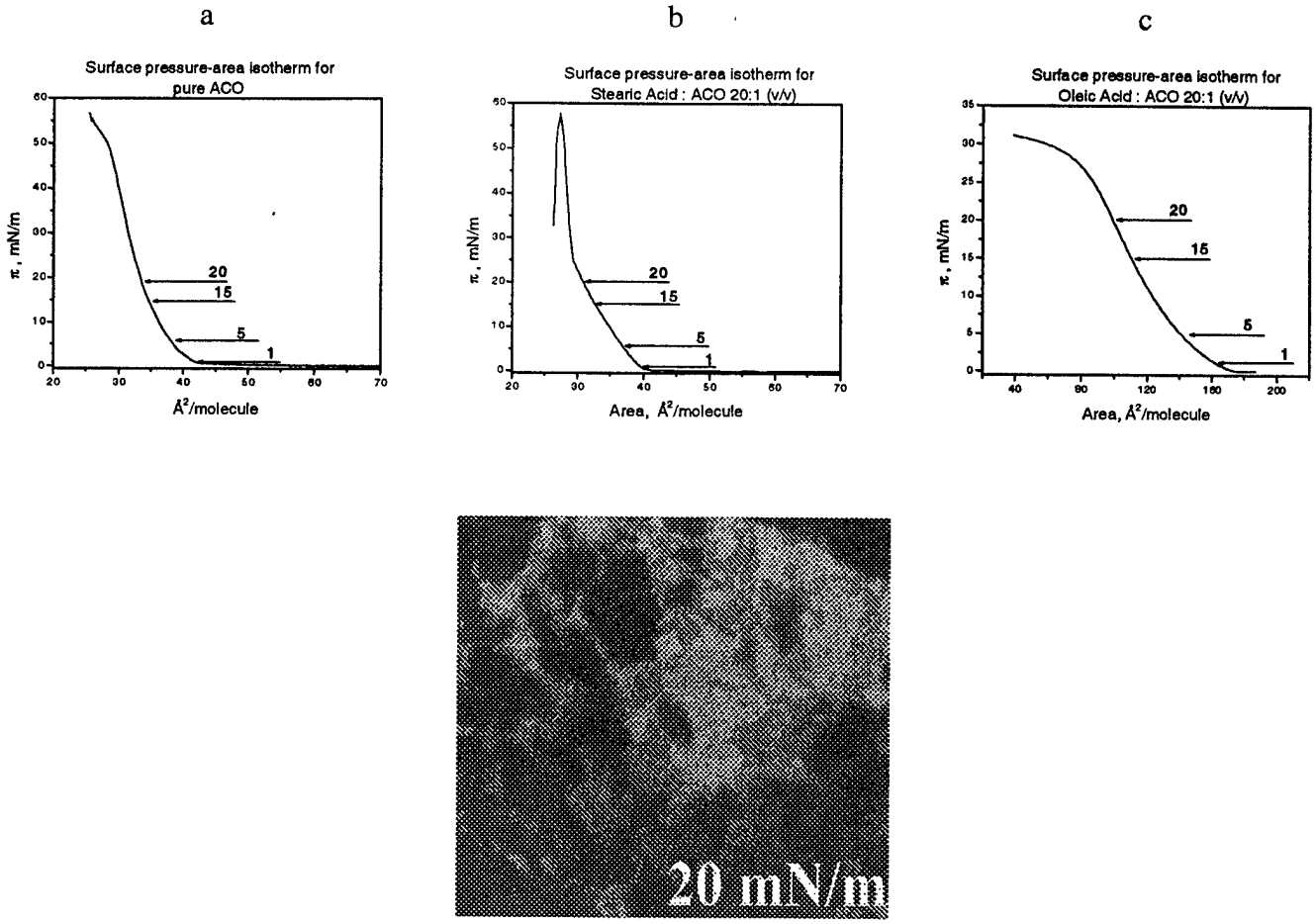

Figure 2: Epifluorescence microscope image of pure ACO monolayer (image size: 895 μm × 713 μm).

(3) List of all publications and technical reports supported under this grant or contract. Provide the list with the following breakout, and in standard format showing authors, title, journal, issue and the date:

(a) Papers published in peer-reviewed journals

NIL

(b) Papers published in non-peer-reviewed journals or in conference proceedings

NIL

(c) Papers presented at meetings, but not published in conference proceedings

NIL

(d) manuscripts submitted but not published

Kele, R., Orbulescu, J., Mello, S., Mabrouki, M. and R.M. Leblanc (2001). "Langmuir and Langmuir-Blodgett film characterization of a new amphiphilic coumarin derivative. Langmuir (in press).

(e) Technical reports submitted to ARO

NIL

(4) Scientific personnel supported by this project and honors/awards/degrees received during this reporting period:

Roger M. Leblanc Principal Investigator

(5) Report of inventions (By Title only):

NIL