

CHAPTER 3

EXPERIMENTAL METHOD

3.0 Introduction

In this section we will discuss the experimental method and materials that used in detail. First a brief introduction to the NIMA Langmuir Blodgett trough that was used in this experiment is described. General precautions when using the LB trough were also detailed. Next the material used and the preparation of different concentration of bR suspensions and the forced mixing of bR and Hexane spreading solutions were also explained.

3.1 Langmuir Blodgett Trough

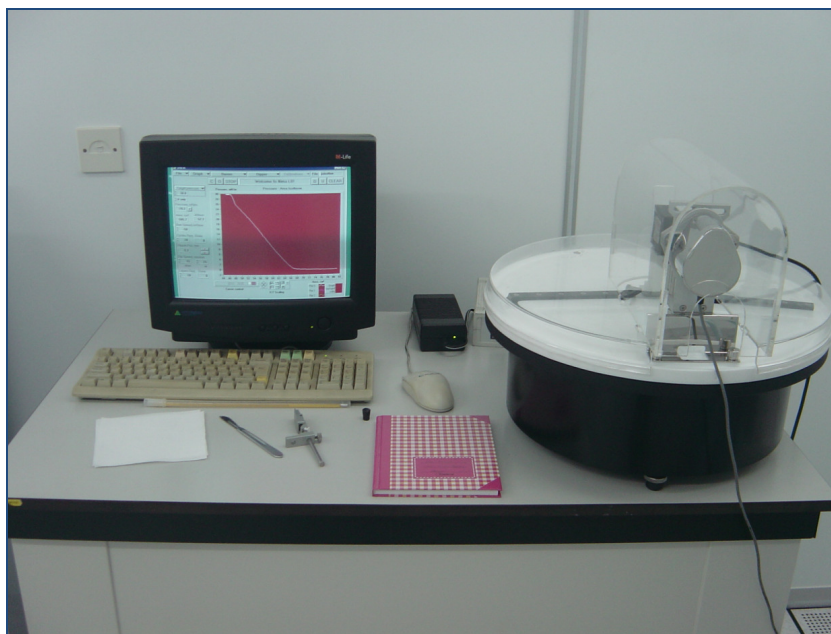


Figure 3.1: NIMA Langmuir Blodgett Trough model 2200

Figure 3.1 above shows the Langmuir Blodgett trough that is used in this experiment. It is a circular type model 2200 from NIMA Technology UK. The trough has two independently movable barriers and a mechanically coupled dipper. The pressure sensor

on this trough uses the Wilhelmy plate to measure the surface pressure. The barriers, pressure sensor and the dipper are all fully automated and computer controlled. This allows the Langmuir film to be studied by simultaneously applying hydrostatic compression. The floating film is compressed uniformly from all sides and the decrease in the area versus the increase in the surface pressure is automatically calculated and plotted by the NIMA 516 software. The precise geometry of the trough consists of two semicircles with radius 180mm and is separated by a 60 mm wide rectangular section in the middle. The dipping well has a dimension of 65(h) X 105 X 85 mm to allow deposition of film onto solid substrate through the vertical dipping method. The trough also comes with two transparent polycarbonate cover that covers the entire trough. This prevents the water (sub-phase) surface from air borne particle contamination. In depth technical details on the operation of the LB trough and the surface pressure sensor can be found in the manual, as such we will deem that the introduction given here is sufficient.

3.2 Langmuir Blodgettry General Precautions

When studying mono-molecular layers, even a small amount of contaminant can cause serious errors. As such cleanliness is the utmost important aspect of Langmuir-Blodgettry. To reduce contamination, the following steps were followed throughout our experiment.

- The entire experiment is conducted in ISO1000 class clean room.
- Proper clean room garment (Jumpsuit with attached hood), face mask, hair net and powder free nitrile gloves were worn.

- The trough is always cleaned using lint free clean room wipe (we used 100% polyester, heat sealed edges wipe) soaked in chloroform before and after each experiment.
- Only freshly prepared de-ionized water is used to fill the trough. If the water needs to be stored for some reasons, only Pyrex glass bottles were used and the water was not be stored for more than 30 minutes.

3.3 Langmuir Blodgett Trough Preparation

Prior to using the LB trough, the trough is horizontally levelled and calibrated. The room temperature is regulated at $20^{\circ}\text{C} \pm 0.5^{\circ}$ and the relative humidity is controlled at 80 %. The trough is cleaned thoroughly using lint free clean room wipe soaked in chloroform. After letting the chloroform dry, the trough is filled up with approximately 1.5 litres of freshly prepared ultra-pure de-ionized water. More water is added or reduced by checking the water meniscus is roughly about 2 mm above the brims of the trough. The water used as subphase was filtered and de-ionized by the use of Barnstead Nanopure system ($18\text{M}\Omega\text{cm}^{-1}$). For the pressure sensor, a Wilhelmy plate with dimensions of 25mm x 10mm is cut from a filter paper a shown in Figure 3.2. A fresh filter paper is used for every isothermal experiment.

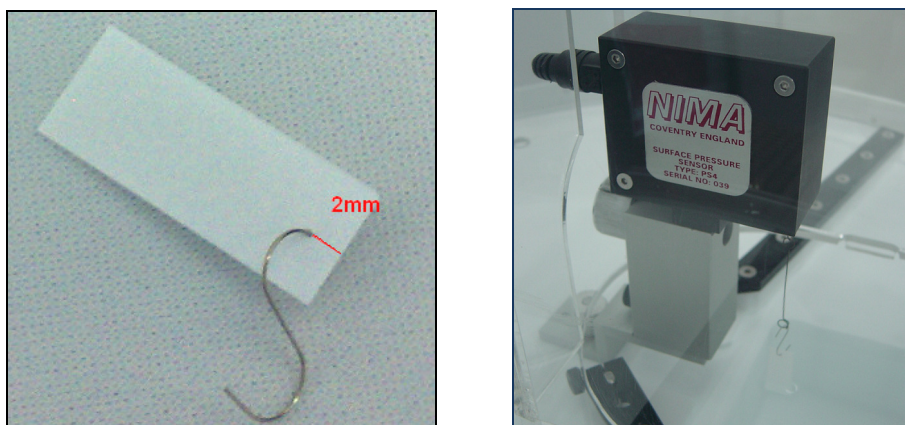


Figure 3.2: Wilhelmy Plate made with Filter Paper and Nima Tensiometer

The filter paper is suspended to the NIMA pressure sensor, through which the surface pressure is fed back to the computer.

Once the surface pressure reading is stabilized, it is reset to zero and the isothermal compression is started. As the barriers reached its minimum area the surface pressure is observed for any increase. Surface pressure of pure water should read as a zero value. Any increase in the surface pressure indicates presence of surface contamination and is removed by a hand held aspirator. This process is repeated until the surface pressure difference of less than 0.3 mN/m is achieved. Once this is achieved, parameters such as concentration, molecular weight and volume are keyed in into the software. Isothermal experiment is then carried out as intended (using different concentration of bR suspension and at various temperatures).

For the experiments where deposition of film is required, the ITO slide (with $15\Omega\text{ cm}^{-2}$) is cleaned in ultrasonic bath for 5 minutes, rinsed with Isopropyl alcohol and then blow dried using compressed Nitrogen gas. Since only one side of the slide is coated with ITO film, two slides were attached together with the surface coated with ITO film facing outside. In this way we could coat two slides on each experiment and save the depositing material. The slides were then attached to the dipper and dipped into the trough prior to spreading the solution.

3.4 Preparation of Bacteriorhodopsin (bR) Suspension

Bacteriorhodopsin(bR) in the lyophilized (freeze dried) form of Variant type D96N from *Halobacterium salinarum* were purchased from Sigma Aldrich. The vendor data sheet states purity of 99.9% and above with a molecular weight of 26784. A bR suspension of 1.0 – 9.0 mg/ml concentrations were prepared by adding bR into pure de-ionized (DI) water. Table 3.1 shows the actual weight and volume of DI water that was used to prepare the different concentration of suspensions.

Table 3.1: Weight of bR and volume of DI water used to prepare different concentration

Weight of bR (mg)(±0.1)	Volume of DI water (ml)(±0.005)	Concentration (mg/ml)(±0.1)
1.5	1.5	1
1.5	0.5	3
2.5	0.5	5
3.8	0.55	7
4.7	0.52	9

Since our experiment deals with mono molecular level studies, even a minute error in the measurement could produce large errors. Though it is difficult to measure the weight of bR and the volume of DI water precisely, all measures must be taken to reduce the errors as much as possible. Weighing of bR is done on the milligram weighing machine. To increase the accuracy of weighing, instead of using the weighing paper to weigh the material and then transfer it to the glass cuvette, the bR was directly put into the glass cuvettes and then weight. Prior to this, the glass cuvette were washed in the ultrasonic cleaner for six minutes and then blow dried using compressed Nitrogen gas. Powder free nitrile gloves were used to prevent contamination from hand

(perspiration and sebum) from sticking to the glass cuvette which could contribute to measurement error.

Freshly prepared DI water is then measured using micro syringe and mixed to the bR. The mixture was then agitated using Snijders Ready to Mix Agitator for 5 minutes for 3 times with intervals of 5 minutes. Agitation was repeated for 3 or more consecutive days until a homogenous bR suspension is produced. bR is a natural protein and may easily denaturalize. As such, the mixture was kept in the refrigerator at freezing temperature during the interval and when ever not in use. The above mentioned method of agitating the mixture for not more than 5 minutes continuously is followed as we do not know the effect of prolonged agitation to the delicate bR molecules. Also sonication is avoided for the same reason. The bR suspension at various concentration is shown in Figure 3.3.

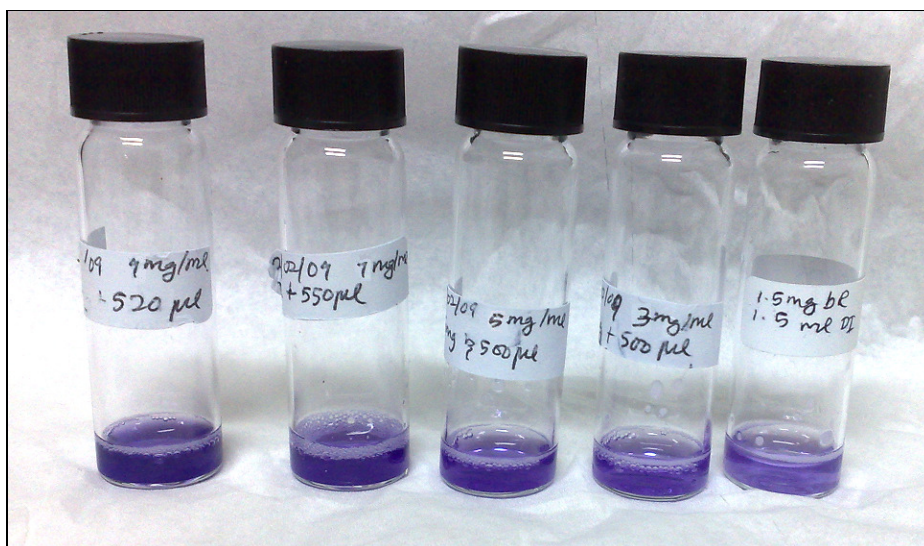


Figure 3.3: bR suspension at various concentration

3.5 Preparation of bR-Hexane Spreading Emulsion

The spreading solution of bR-hexane emulsion is prepared by adding reagent grade hexane to the bR suspension in the ratio of 1:1. Since one of the objectives of this experiment is to see the effect of concentration on the isotherm produced, different volume is used for different concentration of bR suspension. This is to ensure that the same number of bR molecules is spread on the water surface. Table 3.2 shows the volume of bR suspension used for different concentration and corresponding number of molecules spread on the water surface.

Table 3.2: Volume of bR suspension used to prepare spreading solutions and number of bR molecules spread

Concentration of bR suspension (mg/ml)(±0.1)	bR suspension Volume (µl)(±5)	Number of bR molecules spread(± 5%)
1	300	327138718
3	100	327138718
5	60	327138718
7	45	343495654
9	35	343495654

To get the same number of molecules, the actual volume that should have been used for concentration 7 and 9 mg/ml is 42.85 and 33.33 µl respectively. However since the smallest division on the micro syringe is only 5 µl, we have opted to use volume of 45 and 35 µl respectively.

The required volume of bR suspension for the corresponding concentration is transferred into a clean test tube. Same volume of hexane is then transferred in to the

same test tube. Figure 3.4 shows the colourless hexane floating on the purple coloured bR suspension. Forced mixing them was then carried out using a micro-syringe by simply syringing in and out the solutions few times, until a homogenous whitish purple hexane-bR emulsion is produced as shown in Figure 3.5.

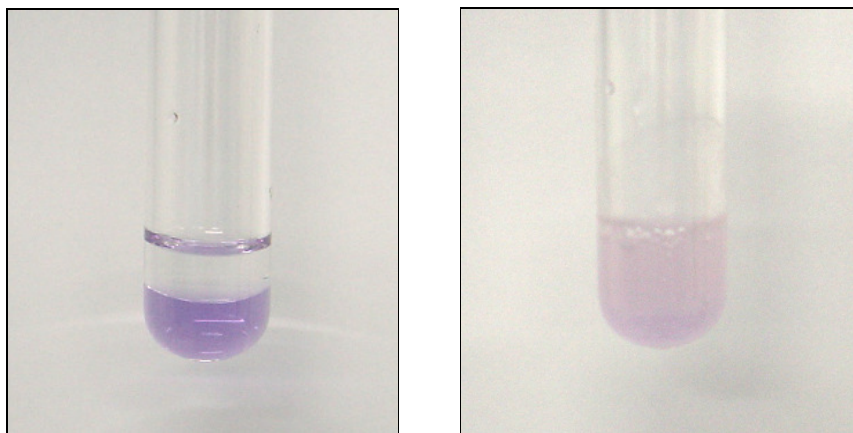


Figure 3.4: bR and Hexane before mixing **Figure 3.5:** bR and Hexane after mixing

The bR-hexane emulsion was then transferred drop by drop onto the water surface in the LB trough. It can be observed that the hexane-bR solution spreads spontaneously on the water surface. The spread solution is left to stand approximately for 15 minutes for the hexane to evaporate [59]. The bR film is then compressed to an annealing pressure of 20 mNm and held there for 20 minutes. The barrier speed is fixed at 20 cm²/min throughout the experiment. The film is then fully decompressed by opening the barriers to its initial area. The film is held at this fully decompressed state for 5 minutes before recompressing the film to achieve full isotherm. The surface pressure-area graph is automatically plotted by the NIMA TR516 software. The above mentioned process is repeated for various concentration of bR suspension as shown in Table 3.1. The surface pressure-area graph for both annealing process and the full isothermal compression are plot on a same graph for each concentration and the results were discussed.

To determine the thermodynamic properties of the bR film, the experiment was repeated at 3 different temperatures of 15, 20 and 25° C (+/- 0.5° C). From the isothermal analysis, various deposition pressures were identified for film deposition. For the film deposition process, an ITO slide that was dipped into the trough prior to spreading the bR suspension is simply withdrawn from the trough at constant predetermined pressure and speed to achieve Transfer Ratio of 1. Once the film deposition is completed, the slides were removed from the trough and air dried for 5 minutes. The deposited film is then subjected to spectroscopic studies using a Jasco V-570 UV-Vis spectrometer to conclude the retention of bR's intrinsic light sensing properties.

The films were also subjected to various microscopic studies such as Atomic Scanning Electron Microscope(SEM), Auger Electron Microscope (AEM) and Surface Profiler to conclude the film thickness and molecular order at various surface pressure. Since the bR monolayer films are deposited onto an ITO slide, and ITO is an electrically conducting material, we did not do any coating (gold, carbon, etc) onto the deposited film to perform the SEM or AEM scanning.