CHAPTER II

MATERIALS AND METHODS

Materials

1. Chemical Reagents.

   1.1 Lidocaine Hydrochloride (Astra, Sweden)

   1.2 Bupivacaine Hydrochloride (Bofors, Sweden)

   1.3 Egg Lecithin (Phosphatidylcholine (E.Merck)

   1.4 Cholesterol (E. Merck)

   1.5 Bovine Serum Albumin (Calbiochem)

   1.6 Disodium Hydrogen Phosphate (Mallinckrodt)

   1.7 Hydrated Monosodium Phosphate (Carlo Erba)

2. Solvents.

   2.1 n - Hexane (BDH)

   2.2 Tridistilled Water. (Goverment Pharmaceutical Organization)

   2.3 Absolute Alcohol USP

3. Apparatus.

   3.1 Torsion Balance (Biolar Coorporation)

   3.2 Teflon Coated Trough with Movable Barrier (CAHN Instrument)

3.3 Micrometer (Wellcome Reagent Ltd.)

3.4 All Glass "Angla" Micrometer Syringe (Wellcome Reagent Ltd.)

3.5 Suction Pump (Arther H. Thomus Coorporation)

3.6 Alcoholic Lamp.

3.7 Plastic Cover.

Methods

1. Preparation of Spreading Solutions

Spreading solutions were freshly prepared before using. In order to form monomolecular film on the full trough surface area, each spreading solution was prepared as follows:

1.1 Egg Lecithin Solution

If egg lecthin (39.8 mg/100 ml.) was dissolved in n - hexane and 0.07 ml of this solution was spreaded on water subphase contained in a 315×10^{16} A^{o2} surface area trough, it would form monomolecular film (Weiner and Rosoff., 1972). For this experiment the full trough surface area is 6.08×10^{17} A^{o2}, so 0.0053774 mgs. of egg lecithin are used to form a monomolecular film. If X mgs of egg lecithin are dissolved in n - hexane to make the X mg/25 ml solution, $\frac{0.1344350 \text{ ml.}}{X}$ of this solution will be used to form monomolecular film by unsing all glass "Agla" micrometer syringe (Fig.4) for spreading. The scale on micrometer shows the distance which micrometer spindle is pushed into the barrel. The spreaded volume is then calculated from this equation.

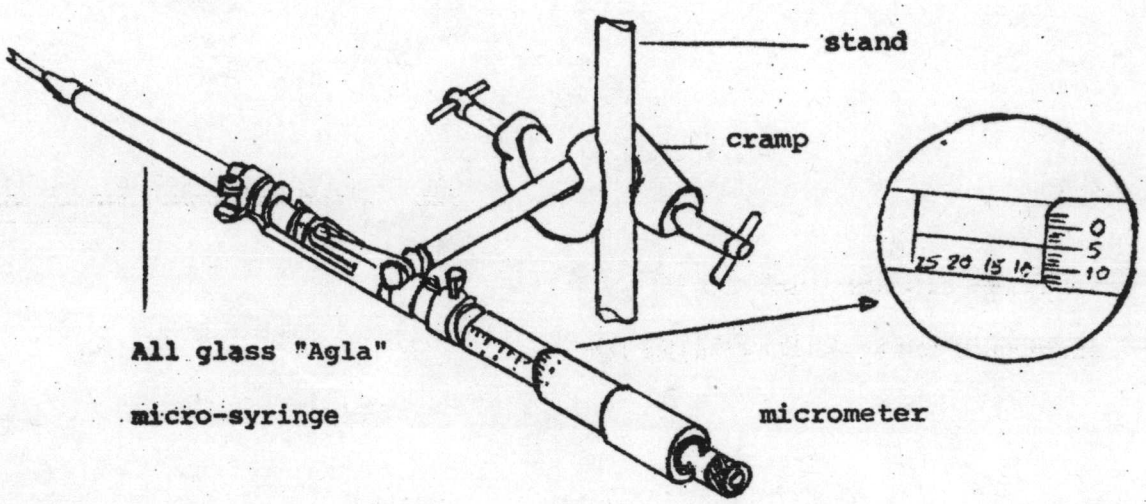

Fig. 4 All glass "Aglass" micro-syringe and micrometer

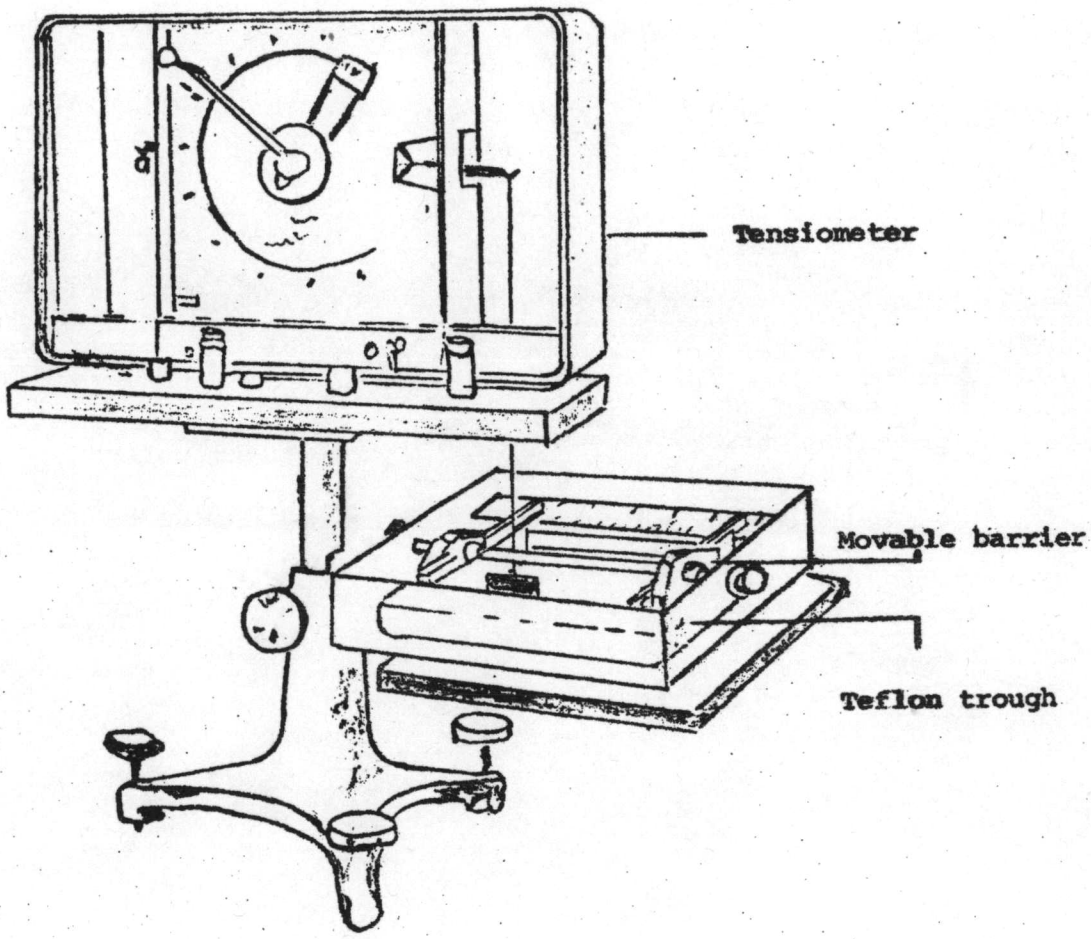

Fig. 5 Tensiometer with teflon trough and movable barrier

20 M = V

M = Micrometer scale (Division)

V = Spreaded Volume (Ml.)

The lecithin solution is therefore used $\frac{0.134435 \text{ ml}}{X}$ or $\frac{6.72175}{X}$ divisions of the micrometer, when X is mg., weight of egg lecithin dissolved in n - hexane to make X mg/25 ml solution (Tantisira,1977).

1.2 Cholesterol Solution

If cholesterol (28.9 mg/100 ml.) was dissolved in n - hexane, 0.07 ml of this solution would be spreaded on the subhphase to form monomolecular film contained in the 315 X 10^{16} $Å^2$ area trough (Weiner and Rosoff,1972). Similarly to # 1.1 if cholesterol solution (X mg/25 ml) is prepared, the volume of this solution to form film on the subphase contained in the 6.08 X 10^{17} $Å^2$ surface area trough of this experiment will be $\frac{4.880875}{X}$ divisions of the micrometer. (Tantisira,1977).

1.3 Bovine Serum Albumin Solution

If bovine serum albumin (45 mg/100 ml) was dissolved in tridistilled water, 0.07 ml of this solution would be spreaded to form monomolecular film on the subphase contained in the 315 X 10^{16} $Å^2$ surface area trough (Weiner and Rosoff,1972). Similary to # 1.1, if the bovine serum albumin solution (X mg/25 ml.) is prepared, the volume of this solution to form monomolecular film on the suphase contained in the 6.08 X 10^{17} $Å^2$ surface area trough of this experiment will be $\frac{7.6}{X}$ divisions of the micrometer. (Tantisira 1977).

2. Preparation of Sorensen's Phosphate Solution

2.1 Monobasic Sodium Phosphate Solution

Monobasic Sodium Phosphate, Anhydrous	8.00	g.
or Monohydrate	9.208	g.
Tridistilled Water q.s	1000	ml.

2.2 Dibasic Sodium Phosphate Solution

Dibasic Sodium Phosphate, Anhydrous	9.47	g.
Tridistilled Water g.s	1000	ml.

2.3 Buffer Solution, pH 5.9

Mixed 90 ml of monobasic sodium phosphate with 10 ml. of dibasic sodium phosphate solution to obtain buffer solution pH 5.9

2.4 Buffer Solution, pH 7.2

Mixed 30 ml of monobasic sodium phosphate solution with 70 ml. of dibasic sodium phosphate solution to obtain buffer solution, pH 7.2

3. Preparation of Local Anaesthetic Solutions.

The following local anaesthetic solutions were prepared by dissolving the substances in prepared buffer solutions.

3.1 0.25% Lidocaine Hydrochloride Solution, pH 5.9

3.2 0.25% Bupivacaine Hydrochloride Solution, pH 5.9

3.3 0.5% Lidocaine Hydrochloride Solution, pH 5.9

3.4 0.5% Bupivacaine Hydrochloride Solution,pH 5.9

3.5 0.25% Lidocaine Hydrochloride Solution,pH 7.2

3.6 0.25% Bupivacaine Hydrochloride Solution,pH 7.2

3.7 0.5% Lidocaine Hydrochloride Solution,pH 7.2

3.8 0.5% Bupivacaine Hydrochloride Solution,pH 7.2

4. Method of Measuring Surface Tension and Surface Pressure

4.1 Measure Standard Surface Tension of

4.1.1 Tridistilled Water

4.1.2 Buffer Solution, pH 5.9

4.1.3 Buffer Solution, pH 7.2

By using surface tensiometer which consists of torsion balance (Fig. 5) with platinum blade, the surface of each subphase contained in the trough was checked triple at each position for cleanliness before measuring the surface tension. The reading of surface tension at the maximum and the two - thirds of the trough area must be equal.

4.2 Determination of Surface Pressure of Monomolecular Films

Surface pressure (π) is the difference between surface tension (r_o) of the subphase and that of the film covered subphases (r)

$$\pi = r_o - r$$

π = Surface Presaure (dyne/cm.)

r_o = Surface Tension of Water Subphase

r = Surface Tension of Film Covered Subphase

4.2.1 Mixed Egg Lecithin - Cholesterol Film Forming

The surface was checked for cleanliness before spreading the film. The solutions of phospholipid (egg lecithin) and cholesterol were spreaded on the subphases by the aid of an "Agla" micrometer syringe. The mixed film was allowed to stand fifteen minutes for complete evaporation of the spreading solvent. Then the surface tension readings were taken at the different trough area by the use of movable barrier until the film was broken. The surface pressures were also determined.

4.2.2 Determination of Surface Pressure of Mixed Egg Lecithin - Cholesterol Films

The surface pressure of mixed egg lecithin - cholesterol films, composed of various surface area ratios (1;3,2:2 and 3:1), were consecutively determined on the different subphase conditions as following :

4.2.2.1 The subphase containing no local anaesthetic at pH 5.9

4.2.2.2 The subphase containing no local anaesthetic at pH 7.2

4.2.2.3 The subphase containing local anaesthetics mentioned in#3.

4.2.3 Mixed Egg Lecithin - Cholesterol - Bovine Serum Albumin Film Forming

The surface of subphases were also checked for the impurity. The bovine serum albumin was added after spreading egg lecithin and cholesterol on the subphase for fifteen minutes. The mixed film was permitted to stand fifteen minutes for equilibrium.

4.2.4 Determination of Surface Pressure of Mixed Egg Lecithin – Cholesterol - Bovine Serum Albumin Films

The surface pressure of mixed egg lecithin - cholesterol - bovine serum albumin films, composed of various surface area ratios (1:3:4, 2:2:4 and 3:1:4), were consecutively determined on the different subphase conditions as following :

4.2.4.1 The subphase containing no local anaesthetic at pH 5.9

4.2.4.2 The subphase containing no local anaesthetic at pH 7.2

4.2.4.3 The subphase containing local anaesthetics mentioned in # 3

The measurement of surface tension was done triple at each area position and under temperature 25° ± 2° c

4.3 Surface Pressure -Per cent of Trough Area Curves

Surface pressure values of each system were poltted against per cent of trough area for comparison and interpretation.