

MATERIALS AND METHODS

1. Materials.

- 1.1 Dexamethasone U.S.P. XVIII (Roussel UCLAF)
- 1.2 Cetylpyridinium chloride (Merrell National L&S Division Cincinnati Ohio).
- 1.3 Benzalkonium chloride (Imperial Chemical Industry)
- 1.4 Absolute alcohol (The Government Pharmaceutical Organization)
- 1.5 White Ointment U.S.P. XIX
- 1.6 Hydrophilic Petrolatum U.S.P. XIX
- 1.7 Hydrophilic Ointment U.S.P. XIX
- 1.8 Polyethylene Glycol Ointment U.S.P. XIX
- 1.9 Chloroform B.P. (Dow Chemical Co.)
- 1.10 Tetramethylammonium hydroxide solution G.R. (E. Merck)
- 1.11 Tetrazolium blue G.R. (E. Merck)
- 1.12 Absolute methanol G.R. (E. Merck)

2. Equipments

- 2.1 Spectrophotometer Pye Unicam Model SP 1800
- 2.2 Cellulose membrane D-2130 (Cellulose dialyzer tubing. Arther H. Thomas Co.)
- 2.3 Electric mixer (Vir Tis)
- 2.4 Constant-temperature water bath (Colora)
- 2.5 Single pan balance (Sauter)

3. Methodology3.1 Preparation of Ointment

- 3.1.1 Reference base. Prepare the following ointment bases to be used as vehicle. White Ointment U.S.P. XIX (Oleagineous Base), Hydrophilic Petrolatum U.S.P. XIX (Absorption Base), Hydrophilic Ointment U.S.P. XIX (Emulsion Base O/W), and Polyethylene Glycol Ointment U.S.P. XIX (Water Soluble Base)

3.1.2 Ointments containing dexamethasone 0.5 mg/g (0.05% W/W). They were prepared by incorporating dexamethasone in fine powder with ointment bases from 3.1.1 on slab using a spatula until a smooth and homogeneous ointment was obtained.

3.1.3 Ointment containing dexamethasone 0.5 mg/g in 2,5,8,11 and 13% water. Dissolved dexamethasone in distilled water (2,5,8,11 and 13%), and incorporated this solution with ointment base from 3.1.1 by using the same technique.

3.1.4 Ointment containing dexamethasone 0.5 mg/g in 2,5, 8,11 and 13% alcohol. These ointment were prepared the same as 3.1.3 but using alcohol instead of distilled water.

3.1.5 Ointment containing dexamethasone 0.5 mg/g in cetylpyridinium chloride 1 : 1000 aqueous solution (2,5,8,11 and 13%). Prepared the same as 3.1.3.

3.1.6 Ointment containing dexamethasone 0.5 mg/g in benzalkonium chloride 1 : 10,000 aqueous solution (2,5,8,11 and 13%). Prepared the same as 3.1.3.

3.2 Release from Ointment

The technique used in this study was diffusion technique, according to method developed by Botarri et al (1,3,6,7,14,20,) by using diffusion cell. The capacity of the cell was 15.90 cm^2 . (Figure 3). The cell was filled with an ointment; the excess was removed by spatula to produce an even surface; and the membrane, which had been soaked in water, was carefully placed and pressed on the ointment. A new membrane was used for each release run. The upper part of the cell was then assembled, thus securing the membrane in place. The filled cell was placed in a beaker (internal height 11 cm, internal diameter 8 cm.) immersed in a circulating constant temperature bath (37°C), The solution outside the cell was stirred using slow speed. The system was designed to produce reasonably fast release rates and to avoid absorption of dexamethasone by the cell material. Prewarmed tridistilled water (37°C , 150 ml) was introduced into the beaker, into which the cell was immediately placed, and stirring was initiated

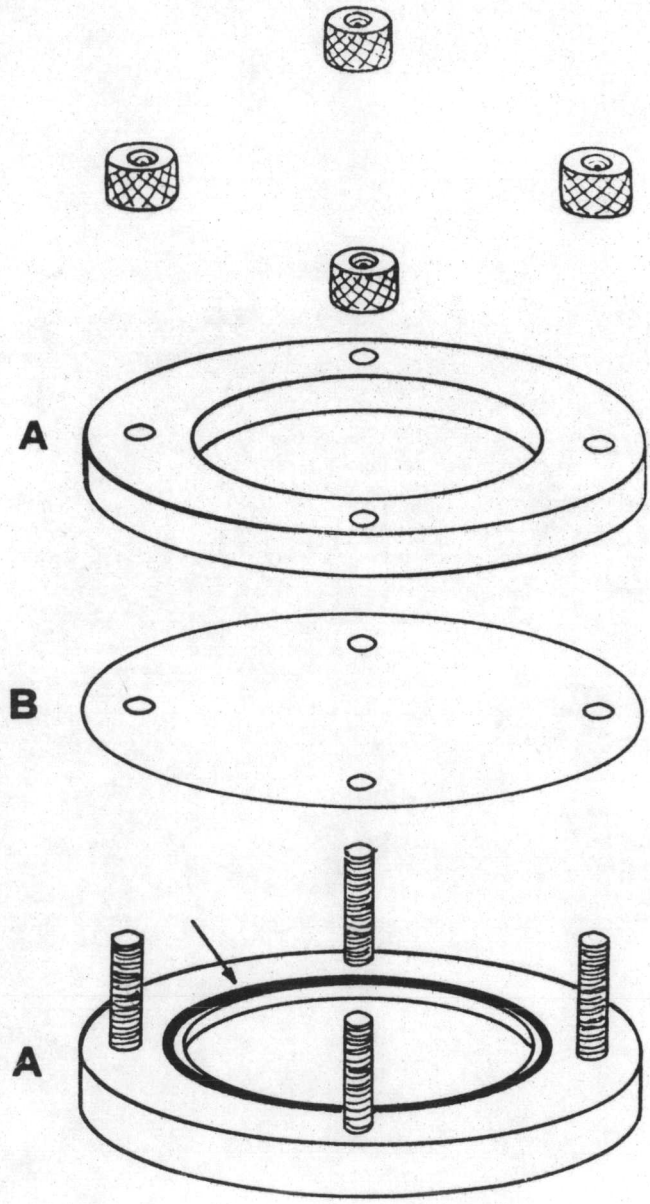


Figure 3 Diffusion cell used for release experiments.

Key : A plastic cell body and plate.

B cellulose membrane

at 15 minutes time intervals for one and a half hours. Pipette 25 ml sample from the beaker to a separatory funnel and replaced at once with an equal amount of prewarmed tri-distilled water. Care was taken to avoid stirring or agitation with the sampling pipette.

3.3 Analytical Method

The analysis of the dexamethasone released during the in vitro test was carried out by U.S.P. XIX method.(17) Aliquot portion of sample solution were extracted by shaken with 25 ml of chloroform for 5 minute-intervals for three times. Three portions of extracted chloroform were combined in an evaporating dish, and were evaporated until dry. Dissolved the dried residue in 20 ml of absolute alcohol, transferred this solution to a 50 ml volumetric flask, reserved as an assay preparation.

Blank run, using plain ointment without drug, to fill the diffusion cell and proceeded the same procedure as previously done. Reserved this solution as a blank.

From 20 ml of assay preparation and blank, add 2 ml of solution prepared by dissolving 50 mg of bluetetrazolium in 10 ml of methanol, and mixed, Then to each flask 2 ml of mixture of 1 volume of tetramethylammonium hydroxide T.S. and 9 volume of methanol was added, mixed, and allowed to stand in the dark for 90 minutes. Read the concentration of dexamethasone in the assay preparation from a spectrophotometer which was set at 525 nm.